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CARL CORRENS

(FRONTISPIECE)

GEHEIMRAT PROFESSOR DOCTOR CARL (ERICH) CORRENS was born in München, Germany, September 19, 1864, the only son of a distinguished painter, ERICH CORRENS and his wife EMILIE CORRENS née Köchlin, also a gifted painter. Orphaned by the early death of both parents,—his father in 1877 and his mother in 1881,—CORRENS was placed in an orphanage at Augsburg in Bavaria, but after spending six months in the gymnasium there his health broke and he was sent to a tuberculosis sanitarium and only a year later resumed his studies in ST. GALLEN, Switzerland, where he was in the care of FRÄULEIN KÖCHLIN, his mother's sister, who saw him successfully through his school years. He graduated from ST. GALLEN with high standing in 1885.

When he entered the UNIVERSITY OF MÜNCHEN he had already a knowledge of general botany and a thorough floristic knowledge of the flowering plants and mosses; but he also had a strong inclination toward chemistry.

At the UNIVERSITY, CARL VON NÄGELI had already ceased to lecture so that CORREN's courses were taken with the Docents, A. PETER, H. DINGLER O. LOEW; but NÄGELI had known CORREN's parents and took an interest in him, advised him in his studies and particularly incited him to study the Algae. The last summer semester of his preparation for the Doctorate was spent at GRAZ with DR. G. HABERLANDT, who took great interest in him. In March 1889 he won his Doctor's degree with a thesis" Ueber Dickenwachstum durch Intussusception bei einiger Algenmembranen," the subject for which had been suggested by NÄGELI. His minor subjects were Physics and Chemistry. After receiving his degree he spent another semester at GRAZ with HABERLANDT, studying the anatomical adaptations of flowers toward visiting insects. Two semesters he was in Berlin in SCHWENDENER's INSTITUT, continuing his studies on the growth of cell membranes; and then for a brief period with PFEFFER in LEIPZIG studying the phenomena of irritability.

In the spring of 1892 he took a position as Privatdozent at the UNIVERSITY OF TÜBINGEN with VÖCHTING, and the same year he was married to ELISABETH WIDMER, a grandniece of NÄGELI. Throughout his scientific career his wife has been his capable and active research assistant as well as the mother of his three children. Under the direction of NÄGELI she had prepared and published (1891) before their marriage a monograph on the European species of Primula.

During his ten years as Privatdozent at TUBINGEN, CORREN'S work was continued chiefly along the physiological lines he had already started, but in addition he began to make use of the botanical garden for experimental cultures. In the middle nineties he began his studies on Xenia, using maize, stocks, beans, peas and lilies. The sharp segregations displayed by the maize kernels on the same cob, and of pea seeds within the same pod, impelled the making of statistical studies which led to the discovery of MENDEL'S laws. With four filial generations of peas before him, he arrived by a sudden inspiration at the correct interpretation, and then made the search through the literature which led to the discovery, with the help of FOCKE's Pflanzen-Mischlinge, that MENDEL had arrived at the same conclusions thirty-five years before.

In the spring of 1902 CORRENS accepted a call to LEIPZIG as Professor extraordinarius. In 1909 he went to MUNSTER I. W. as Professor ordinarius

and in 1913 was appointed first Director of the KAISER WILHELM INSTITUT FÜR BIOLOGIE in Berlin-Dahlem, where he is still active, having established one of the most important centers of genetical research in the world.

The publication of his classic work on Xenia which opened his genetical career in 1899; his independent rediscovery of Mendel's laws, announced in 1900 simultaneously with similar announcements of DE VRIES and TSCHERMAK; and a 200-page paper on maize hybrids published in 1901 placed him at once in a position of leadership which he has maintained consistently ever since. He has not hesitated to attack the most difficult genetical problems, involving sex, variegation, self-sterility, heterostyly, differential pollen-tube growth, experimental modification of sex-ratios, etc.

To celebrate his sixtieth birthday the DEUTSCHEN GESELSCHAFT FÜR VEREBUNGSWISSENSCHAFT collected and published in 1924 in a single volume of about 1300 pages all his genetical papers, sixty in number, except two independent publications on sex.

CORRENS was awarded in 1912 the SÖMMERING medal of the SENCKENBERGISCHEN NATURFORSCHENDEN GESELLSCHAFT, and the same year received the civil Order of the Red Eagle, 4th Class, and in 1915 the title "Geheimer Regierungsrat." Honorary doctorates have been conferred on him in Medicine (ROSTOCK), Agriculture (LANDWIRTSCHAFTLICHE HOCHSCHULE BERLIN," Civics (MÜNSTER I. WESTFALEN), and natural science(TÜBINGEN). He is a Member of the PRUSSIAN ACADEMY OF SCIENCE, Non-resident or Corresponding Member of the SCIENTIFIC SOCIETY IN GÖTTINGEN, the ACADEMIES in Vienna, München and Stockholm, and Corresponding Member or Honorary Member of the DEUTSCHEN GESELLSCHAFT FUR VERERBUNGSWISSENSCHAFT, the BOTANICAL SOCIETY OF AMERICA, the BOTANICAL SOCIETY OF TOKYO, etc., etc.

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A CYTOLOGICAL AND GENETICAL STUDY OF PROGENIES OF TRIPLOID TOMATOES[1]

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INTRODUCTION

Perhaps the most familiar kind of mutation is that which appears to involve a change at a specific locus in a chromosome, commonly called a gene mutation. No change of a quantitative kind is visible in the chromosomes. Mutant characters of this kind were used by MENDEL in his famous "Experiments in plant hybridization." As a general rule, such mutants can be made to breed true. Another kind of mutation is that associated with a change in the number of chromosomes. Usually one or more complete sets of extra chromosomes are present as in polyploids or a smaller number is present in addition to the normal complement. As a rule, except in the case of certain types of polyploidy, these chromosomal mutants are genetically inconstant and cannot be made to breed true.

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Doubtless chromosomal mutants were found by the hybridists and earlier geneticists and, before the advent of the chromosome theory, these must have been a source of much perplexity both from their elusive character and from their peculiar mode of inheritance. Probably in many cases they were thought to be the result of accident or disease. Recent work seems to have brought to light an essential similarity between chromosomal and gene mutations. According to the theory of gene balance which we owe chiefly to BRIDGES (1921), the characteristics of mutants of both kinds are an expression of a certain gene balance. While a gene mutant appears to result from a qualitative change at a certain locus in the chromosome and a chromosomal mutant from a quantitative change occasioned by the change in chromosome number, in both the genes play an essential part in determining the mutant characters.

While a diploid plant contains $2n$ chromosomes, where n represents the number of pairs present in the heterotype division up to the beginning of the anaphase, a triploid contains $3n$ chromosomes, or n sets each consisting of 3 chromosomes which tend to attract one another. According to a recent list (LESLEY, MARGARET M., 1926), triploidy has been reported in Oenothera, Morus, Uvularia, Datura, Canna, hyacinth and tomato; to this, maize (RANDOLPH and MCCLINTOCK 1926), Primula (IINUMA 1926) and Crepis (NAVASCHIN 1926) may now be added. Apparent triploid plants originating from species crosses followed by parthenogenesis as in the polyploid roses studied by TÄCKHOLM (1922) are not necessarily triploids in the strict sense.

A triploid plant is usually a replica of the corresponding diploid but is distinguished by the larger size of the organs, cells and nuclei. Usually the pollen is scanty and contains many empty grains. Little viable seed is produced.

BLAKESLEE (1924) has shown that the progeny of a triploid Datura includes a remarkable series of forms with one or more extra chromosome. Although different in origin, these forms are undoubtedly of the same nature as certain of the mutants which regularly appear among the progeny of normal Datura plants.

A triploid tomato plant which chanced to appear in a commercial variety was the starting point of the present work. As in the case of Datura, crosses between triploid and diploid tomato plants gave rise to a series of variant types comparable with chromosomal mutants. The number of chromosomes has been determined and the characteristics and genetic behavior of certain of these types have been studied with the special object of throwing some light on the composition of the individual chromosomes.

ACKNOWLEDGMENTS

The writer is deeply indebted to his wife, Doctor MARGARET MANN LESLEY, for much help especially in the cytological work. He has also received valuable suggestions from Professor ROY E. CLAUSEN, Division of Genetics, UNIVERSITY OF CALIFORNIA. For advice in the preparation of this manuscript the writer is indebted to Professor E. B. BABCOCK, Division of Genetics, UNIVERSITY OF CALIFORNIA, and to Doctor HOWARD B. FROST, Citrus Experiment Station, UNIVERSITY OF CALIFORNIA.

METHODS

Chromosome number was, as a rule, obtained from smears of pollen mother cells, stained by Belling's method. The most generally favorable stage for counting was the second metaphase but cells in which both plates are countable are sometimes difficult to find; diakinesis was often used and occasionally interkinesis and second anaphase. First metaphase and anaphase often gave useful indications. In exceptional cases to be noted later, chromosomal counts were obtained from sections of root tips and of anthers fixed in chrom-acetic-urea and stained in Heidenhain's iron-alum haematoxylin. In no case was a constant discrepancy found between chromosome number in the root tips and in the pollen mother cells, but the occasional presence of tetraploid pollen mother cells in diploid or trisomic plants previously reported by M. M. LESLEY (1925) was confirmed. Although the chromosomes are only about 1μ in diameter at the second metaphase, their relatively small number and their shortness in the later phases of meiosis and in root tips, make the tomato fairly favorable material for chromosome counting.

In many first-metaphase plates there is some gradation in chromosome size; in particular one large bivalent is often seen at the periphery and smaller ones in the center of the plate. These differences, however, are not constant and are probably due to slight differences in stage of development; they were not detected in root tips.

The pollen was examined in acetocarmine. Mixed samples were taken from what appeared to be typical healthy and ripe anthers. The grains which were round and full of stained material were counted as "good" and those which were irregular in outline, imperfectly filled or empty were counted as "bad." Very few burst grains were found. In the same plant and even in individual anthers of the same flower the variability both in quantity of pollen and in proportion of good grains greatly exceeded that of random sampling.

Unless otherwise stated, all flowers in both self- and cross-pollination were inclosed in paper bags and the usual precautions taken in pollination. Field cultures were for the most part grown in the experimental grounds of the Division of Genetics, at Berkeley, and greenhouse cultures at the CITRUS EXPERIMENT STATION, Riverside.

ORIGIN OF TRIPLOIDS AND F_1 PROGENY

The normal tomato plant was found by WINKLER (1910) to contain 24 chromosomes in the root tips and 12 pairs at diakinesis in the pollen mother cells. In 1923 a single triploid plant was found among 29 plants of Livingston's Dwarf Aristocrat, grown from commercial seed. The root tips contained 36 chromosomes and a study of the prophases of meiosis in the pollen mother cells by M. M. LESLEY (1926) showed at diakinesis, as a rule, 12 trivalents. The morphological characteristics of this plant have been briefly described in a previous paper (LESLEY and MANN 1925). The stems, leaves and flowers were more or less gigantic but the fruits were undersized and few in number. In 1924 a second triploid plant identical in appearance with the first occurred among 85 plants from the same lot of seed (figure 3). A third appeared among 104 plants also from commercial seed of a rather similar variety, Dwarf Champion, and a fourth in a commercial planting of an early strain of Stone, a variety of standard habit. Altogether among 200 plants of Dwarf Aristocrat 2 plants (1 percent) and among 280 plants of Dwarf Champion 1 plant (0.4 percent) were triploid. Some of the giant unfruitful plants known to commercial tomato growers as "he" plants or "fruitless hybrids" are undoubtedly triploids, others perhaps are trisomics as is noted below (p. 36).

A triploid plant may originate from a cross between a tetraploid and a diploid. Tetraploid tomato plants were obtained by WINKLER (1916) from shoots arising from the callus of a graft between *Solanum nigrum* and tomato. He reports regular 24+24 reduction in the pollen mother cells but only 5 percent of good pollen. No tetraploid plant has been found in the course of the present study. Recently M. M. LESLEY (1925) has found islands of tetraploid cells in roots and groups of tetraploid pollen mother cells which are believed to have originated from tetraploid islands in the meristems of shoots. She also found diploid pollen mother cells of tomato in which the reduction division was suppressed with resulting dyad formation (unpublished data). In each case a diploid gamete would be formed, which, when fertilized by a normal (haploid) gamete, would give a triploid plant. Probably triploids have arisen in both ways.

The two triploid plants of Dwarf Aristocrat used in practically all the breeding work showed the dwarf habit, rather dark green foliage and smooth scarlet fruit characteristic of this variety. Numerous attempts were made to obtain seed from self-fertilization but without success. Seeds from a few fruits which set on one plant late in the season without hand-pollination gave rise to plants of standard habit and therefore resulted from vicinism. Seed was produced very sparingly from diploid ♀ × triploid ♂, and therefore the failure to obtain seed from self-fertilization seems to be due to defective pollen. Indeed very little mature pollen is found in the anthers of the triploid and a very large proportion of the grains are empty or clearly degenerate (table 5). Triploid Datura produced very little good pollen but gave some progeny from self-fertilization. Seed was readily obtained from triploid tomato ♀ × diploid ♂. In 56 fruits obtained from crosses of this type the mean number of viable seeds per fruit was about 4, as compared with about 50 in diploid Dwarf Aristocrat selfed.

Since pollination with a diploid proved to be the most effective method of obtaining progeny a choice of diploid pollen parents had to be made. Clearly the identification of forms with extra chromosomes was of primary importance. How greatly identification of a trisomic form may be hindered by differences resulting from the segregation of genes has been clearly pointed out by CLAUSEN and GOODSPEED (1924). In the present case, therefore, since self-fertilization had failed, the triploids were crossed with a diploid plant *of the same variety*.

The progeny of triploid × diploid Dwarf Aristocrat will be referred to as the non-hybrid F_1. This term is not strictly correct since the triploid and diploid plants were probably not genotypically identical. The two diploid plants used appeared to be typical of the variety, were similar in appearance and probably belonged to the same pure line, but both may have differed genotypically from the triploids. Among the 10 plants with the diploid chromosome number which appeared in the non-hybrid F_1, the only obvious variation was in foliage color. In the non-hybrid progenies there was rarely any difficulty in identifying healthy diploid plants in the field or greenhouse. It appears, therefore, that the genotypic differences in the non-hybrid progeny were not sufficient to obscure the differences associated with the presence of extra chromosomes. Small hybrid F_1 progenies were also grown from triploid crossed with the varieties Globe, Red Pear, and Magnus, for comparison of the hybrid with the corresponding non-hybrid forms.

The F_1 triploid × diploid progenies contained a most striking diversity of types, varying in plant size, color and shape of foliage, flower structure, fruit shape and other characters. Part of these plants were grown in the field and part in the greenhouse. Certain characteristics, especially of foliage, were more evident in the field than in the greenhouse, so that the identification of types, particularly by foliage, was sometimes difficult (see figures 7 and 8). BLAKESLEE and FARNHAM (1923) report a similar experience with the Poinsettia mutant of Datura.

CHROMOSOME NUMBER OF F_1 PROGENY OF TRIPLOID × DIPLOID

Table 1 shows the chromosome number of the hybrid and non-hybrid progenies of triploid ♀ × diploid ♂. M. M. LESLEY (1926) has shown that in the pollen mother cells of triploid tomato the unpaired chromosomes are distributed at random at the heterotypic division, and consequently it might be expected that the chromosome number in the microspores would show approximately a binomial distribution. Such a distribution was also found by BELLING and BLAKESLEE (1922) in the pollen mother cells of Datura and by BELLING (1924) in hyacinth. It is at least not improbable

TABLE 1

Chromosome number of F_1 progeny of triploid × diploid tomato.

Number of chromosomes	2n	2n+1	2n+1 or 2n+2	2n+2	2n+3
Frequencies observed	7	26[2]	–	7	2
	3[3]	9[3]	5[3]	7[3]	
Total frequencies	10	35	5	14	2

that the chromosome number of the eggs in these triploids has a similar distribution. It is evident from table 1 that, in tomato, the chromosome number of the progeny has quite a different distribution. In a binomial series, 30-chromosome plants would be the most frequent, but no 30-chromosome plant appeared and no gamete with more than 3 extra chromosomes has given a viable zygote. Some of the counts are not decisive but the data certainly warrant the conclusion that the 25-chromosome plant or simple trisomic is the most frequent and suggest that the

[2] Including one plant with additional fragment.

[3] Based on fewer counts.

double trisomic, diploid and triple trisomic are next in order. Since chromosome counts were obtained from only about half the population, these data represent only a sample of the F_1 progeny grown. Probably the frequency of 26- and 27-chromosome plants is underestimated since, as a rule, they produce few or even no buds and relatively few counts were made from root tips. On the other hand a few plants which were obviously diploid were not examined cytologically. One plant was found to contain a whole extra chromosome and an additional fragment.[4] This and other fragment-containing types which occurred in the progeny of double trisomics will be described in another paper. No plant was found to have less than 24 chromosomes.

Presumably the numerous, more or less undersized and abortive seeds which are to be found in the fruit of triploids, represent the inviable combinations with high chromosome number, if indeed the eggs with high chromosome numbers ever became fertilized.

A correlation was found between seed size and chromosome number similar to that observed by FROST (1919) in Matthiola. Non-hybrid F_1 seeds were arbitrarily grouped into "large" and "small." The chromosome numbers of the resulting plants are shown in table 2. The data indicate that "small" seeds give rise to a larger proportion of 26- and 27-chromosome plants. One "large" seed however gave rise to a 26-chromosome plant that was extremely abnormal in development.

TABLE 2

Seed size and chromosome number of F_1 plants from triploid × diploid tomato.

SIZE OF SEED	NUMBER OF SEEDS	DIPLOID (PERCENT)	TRISOMIC (PERCENT)	ONE EXTRA CHROMOSOME[5] (PERCENT)	MORE THAN ONE EXTRA CHROMOSOME[5] (PERCENT)
"Large"	28	21	79	57	14
"Small"	22	5	95	50	32

As a result of numerous attempts, only 2 plants have been obtained from diploid pollinated by triploid. These originated from diploid × triploid Dwarf Champion and both F_1 plants were diploid.

[4] The number of chromosomes in this plant, and in the 27-chromosome and some 26-chromosome plants was determined from root tips by Doctor M. M. LESLEY.

[5] Excluding plants with extra chromosomes of which the number was not positively determined.

Discussion of chromosome number

WINKLER (1916) first suggested that hyperdiploid forms were likely to differ more than tetraploids from the normal on account of unbalance in chromosome number.

In the progeny of Datura triploid×self and triploid×diploid, BLAKESLEE and BELLING (1924) found no plant with more than 3 extra chromosomes, although the distribution of the chromosomes in meiosis in the pollen mother cells was approximately binomial. As all combinations with more than 27 chromosomes were absent, it was concluded that an unbalance exceeding 3/24 was inconsistent with viability. In the progenies of the triploid *Oenothera semigigas*×*O. Lamarckiana* and *O. biennis*, VAN OVEREEM (1920) found plants with chromosome number ranging in unbroken series from 14 (diploid) to 20. In the progeny of *O. semigigas*×mut. *velutina*, DE VRIES and BOEDIJN (1924) found a similar range of chromosome number with a mode in the 15-chromosome class. In this species, unbalance in chromosome number appears to have no obvious relation to viability; this seems to point to a certain similarity of its chromosomes at least in genes affecting viability. Presumably the greatest unbalance in chromosome number in these plants is 3/14 and 4/14, for, if 7 is the basal number, a 20-chromosome plant would be less unbalanced than one with 17 or 18 chromosomes.

A glance at table 1 at once suggests that in tomato, as in Datura, viability has some relation to unbalance of chromosome number and is at least an approximate measure of it. It appears that an unbalance of 3/24 is the maximum consistent with viability and that, as a rule, the greater the unbalance, the less the chance of viability of gamete or zygote. It is probable that 27-chromosome plants occur more frequently in the F_1 from triploid×diploid in tomato than in Datura, since 2 such plants were found among 66 F_1 plants in tomato while BLAKESLEE and BELLING (1924) mention only 1 among a much larger F_1 progeny in Datura. This suggests that Datura is more sensitive to changes of numerical chromosome balance than tomato.

BRIDGES (1923) found in Drosophila that triplo-X or triplo-IV or haplo-IV flies were viable but that no triplo-II or triplo-III appeared in the progeny of triploids. Since the numerical chromosome unbalance is the same in triplo-IV and triplo-III, it appeared that the size of the extra chromosome had to be taken into account. According to BRIDGES (1922) the effect of extra chromosomes on development depends on their internal gene balance. It is inferred that the internal gene balance differs in the

chromosomes of the same and of different species. Except in the cases of polyploidy and of replication of the same chromosome, viability will depend, not on the unbalance in terms of chromosome number, but rather on the gene unbalance of the individual chromosomes and combinations of chromosomes. This hypothesis explains why a gamete of Oenothera can sustain an unbalance of 3/7 but a gamete of tomato probably only an unbalance of 3/12.

BEHAVIOR OF EXTRA CHROMOSOMES

The mode of origin of the triploid × diploid F_1 and the presence of trisomes in meiosis of 25- and 26-chromosome plants suggest that the tomato plants with extra chromosomes are, in BLAKESLEE'S (1921a) terminology, simple, double and triple trisomics. Although double trisomics were less extensively studied than simple trisomics the evidence indicates no great difference in the behavior of the extra chromosomes in these two groups. Neither has any constant difference in the behavior of the extra chromosome been observed in different simple-trisomic types.

In diakinesis the arrangement of the set of 3 chromosomes in the pollen mother cells of a simple trisomic is the same as that found by M. M. LESLEY (1926) in her study of the prophases of the triploid tomato. Either 11 pairs and 1 trivalent, the latter arranged in a single row or in a triangle (figure 2A), are present, or less frequently 12 pairs and 1 unpaired chromosome (figure 1A). Usually one member of the set of 3 chromosomes lags during the formation of the first metaphase plate, as commonly one monovalent is seen outside the plate in lateral view of the first metaphase. Sometimes however, the association typical of diakinesis is maintained and 11 pairs and 1 fairly compact trivalent appear; the latter has sometimes been seen as a compact row of 3 in lateral view. In polar views, accordingly, 12 bivalents and 1 monovalent (figure 2B) or 11 bivalents and 1 trivalent (figure 1C) were found. One or two laggards are common in the early first anaphase (figure 1D). It is probable that the unpaired chromosome usually passes without division into one of the second-metaphase plates, as 12+13 (figure 2C) is more common that 13+13 at that stage. A lagging chromosome is usually included in one of the second-metaphase plates, as it is exceptional to find 12+12 at that stage. Sometimes, however, during interkinesis one or two (figure 2D) micronuclei or karyosomes are seen with membranes and chromatin in a condition similar to interkinesis. In such a case the lagging chromatin doubtless fails to reach the second-metaphase plates and forms microcytes

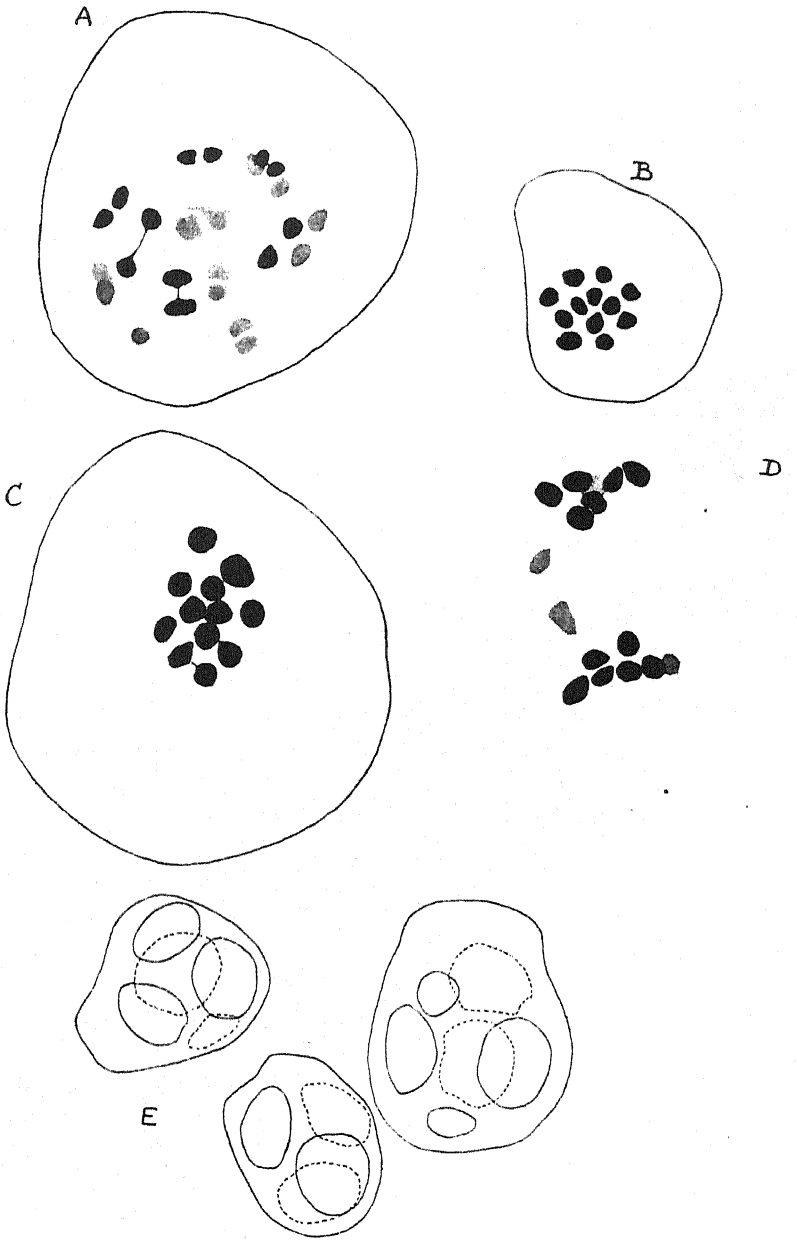


FIGURE 1.—Pollen maturation in diploid and simple trisomics. A, triplo-A, diakinesis, 12 bivalents (pairs) and 1 univalent; B, diploid, 1st metaphase, 12 bivalents; C, triplo-A, 1st metaphase, 11 bivalents and 1 trivalent; D, triplo-E, late 1st anaphase, 2 laggards from division of unpaired chromosome (polar groups not countable); E, triplo-A, tetrad (below), pentad (left), hexad (right). A, C, D×4000; B, E×2200.

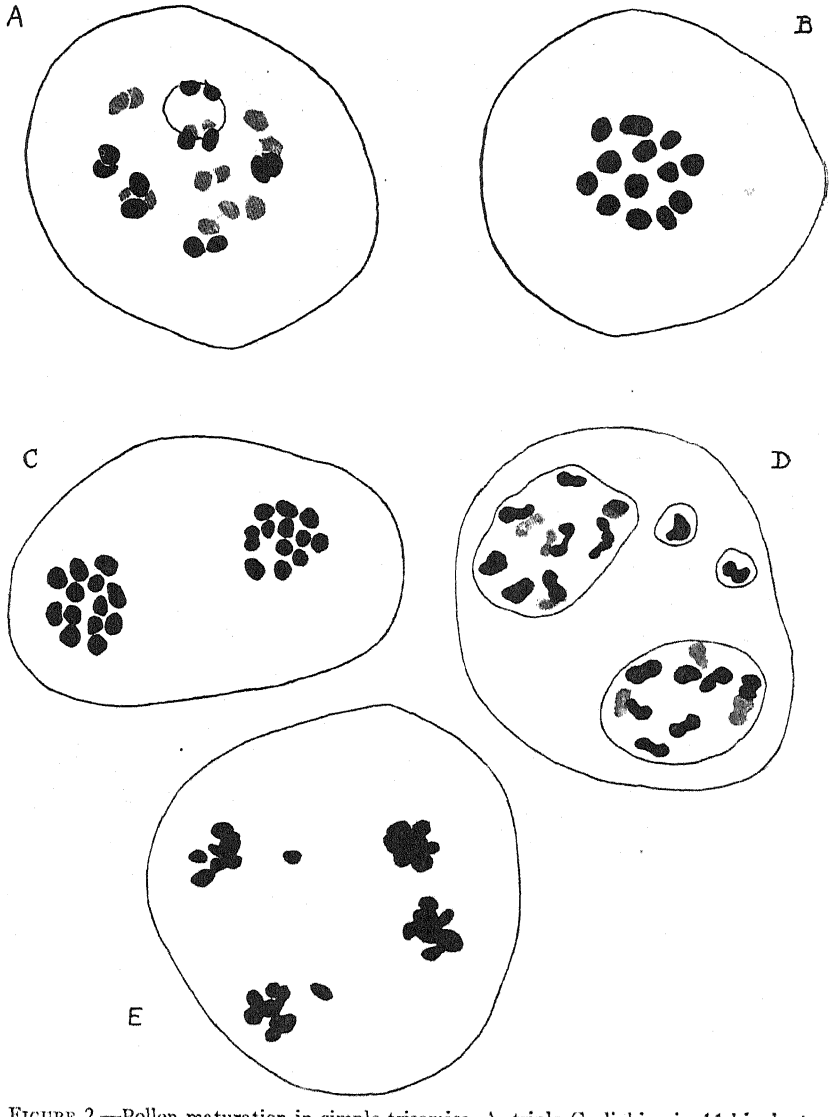


FIGURE 2.—Pollen maturation in simple trisomics. A, triplo-C, diakinesis, 11 bivalents and 1 trivalent; B, triplo-A, 1st metaphse, 12 bivalents and 1 monovalent; C, triplo-C, 2nd metaphase, 12+13; D, triplo-C, interkinesis showing micronuclei (chromosome count not clear); E, triplo-A, ate 2nd anaphase, 2 laggards from division of unpaired chromosome in 1st division. ×3050.,

(figure 1E). In the second anaphase, after assortment in the heterotypic division, the unpaired chromosome divides so that 13+13 and 12+12 result; or after division at the first anaphase, assortment occurs forming 12+13, 12+13. As before, the unpaired elements often lag during the second anaphase and 2 laggards are seen in one plate or 1 in each (figure 2E). Since 12+13 is more common than 13+13 at the second metaphase, it was surprising to find 1 laggard in each second anaphase plate more commonly than 2 laggards in one plate. Apparently lagging in the second anaphase is more common in the process of *assortment* of the 2 unpaired elements than in the *division* of 1 or, in other words, in the second division assortment is slower than the completion of the process of division. It is evident from the above statement that there is much variation in chromosome behavior during meiosis. While the process tends to follow a certain rule there is no constant behavior of the chromosomes even in material prepared on the same day and from the same anther. Such irregularity seems to be characteristic of meiosis where unpaired chromosomes are present.

The microspore groups or "tetrads" of a simple trisomic may contain 1 or 2 microcytes (figure 1E). Owing to the compact arrangement of the microspores, the counting of microcytes was usually made after much shrinkage had been allowed to occur. The occurrence of micronuclei at interkinesis noted above indicates that some microcytes originate in the heterotype division. From 4.5 percent to 7.6 percent of "tetrads" containing microcytes were counted in 3 different simple trisomic types (table 3). Probably the proportion differs significantly in these types although considerable variation was found in different samples from the same type. In the "tetrads" of double trisomics 3 microcytes were often present.

TABLE 3

Microcytes in pollen "tetrads" of trisomic tomatoes.

NAME	NUMBER OF "TETRADS" EXAMINED	PENTADS AND HEXADS, PERCENT
Triplo-A	513	4.5
Triplo-B	426	7.1
Triplo-C	1154	7.6

GENERAL CHARACTERISTICS AND NAMING OF TRISOMICS

All the trisomic plants which have appeared either in the F_1 progeny of triploids or in the progeny of trisomics show certain general characteris-

tics which serve to distinguish them from diploids. They are slower-growing, and more or less distinct in foliage. They have a tendency to pollen sterility and unfruitfulness which is more marked under field than under greenhouse conditions. Although even simple trisomics are distinguished from diploids by their smaller size up to the time when the first fruits set, later in the season certain of them may exceed diploids in plant size, doubtless on account of their unfruitfulness. Most of the trisomic types are later in flowering, show some difference from diploids in the size or structure of the floral organs and produce fewer viable seeds. These peculiarities are in general more marked in double and triple than in simple trisomics, especially the slower rate of growth, pollen sterility and diminished seed production. In the chromosomal mutants of Matthiola, according to FROST (paper in preparation) hybrid forms are much more fertile than non-hybrid. So in tomato, certain double-trisomic hybrids set seed more freely and were probably more vigorous and more fertile than the corresponding non-hybrids. When the trisomic hybrid type can readily be identified, it is therefore preferable to the non-hybrid for breeding work, but the differences introduced by segregation of genes from hybrid parents increase the difficulty of recognizing extra-chromosome types, especially those containing chromosome fragments.

An attempt has been made to point out some of the most conspicuous features in which certain trisomic types differ from the diploid. Only a beginning has been made in the description of these types. Some of the simple trisomic plant types are by no means easy to distinguish from diploids (see figures 6 and 12). In tobacco the discovery by CLAUSEN and GOODSPEED (1924) of the simple trisomic "Enlarged" was made by the use of a correlation table. So in the tomato, the distinguishing characteristics of certain trisomics, such as rate of growth, number of loculi in the fruit, and foliage color are of a quantitative nature and so variable as to make identification difficult to one unfamiliar with the types, especially where only a small number of plants are observed.

In the naming of trisomics, a modification of the method used by BRIDGES (1921) is adopted here. The capital letters of the alphabet are used to distinguish the individual chromosomes. The haploid number of chromosomes in the tomato is 12 but as yet only nine different types each with 1 whole extra chromosome have been found. Accordingly the letters A to I are used to name these 9 chromosomes. A simple trisomic (25 chromosomes) may then be triplo-A, which means that the A-chromosome is present in triplicate, the remainder being made up of 11 normal pairs. A double trisomic may be triplo-AB, which means that the A- and B-

chromosomes are present in triplicate, the balance consisting of 10 normal pairs.

DESCRIPTION OF TRISOMIC TYPES

Since the distinguishing characteristics of trisomic types may be masked in hybrid progenies, the non-hybrid progeny of triploid Dwarf Aristocrat was used as the basis for the following descriptions.



FIGURE 3.—Triploid (left) and diploid (right) plants of Dwarf Aristocrat tomato.

The diploid form of Dwarf Aristocrat shows the characteristics of stem and leaf which combine to make the dwarf habit. The stem is short and stiff, giving the plant a rather erect habit until borne down by the weight of the fruit. The leaves are dark green, thick and leathery in texture and deeply rugose, and the leaflets are nearly sessile on the rachis (figures 5 and 6). The style length is about 5 mm and the top of the stigma is

TABLE 4

Shape and number of loculi ("cells") in fruit of diploid and non-hybrid trisomic tomatoes.
(For explanation of terms, see figure 4, legend)

	NUMBER OF FRUITS MEASURED	MEAN E. D.	MEAN $\frac{\text{E. D.}}{\text{L}}$	MEAN $\frac{\text{L}}{\text{P. D.}}$	NUMBER OF FRUIT CUT	MEAN NUMBER OF CELLS PER FRUIT	STANDARD DEVIATION OF NUMBER OF CELLS
Diploid	47	4.7	1.2	1.2	87	4.4	1.4
Triplo-A	9	5.4	1.4	1.4	8	5.4	
Triplo-C	4	5.2	1.5	1.7	50	8.8	2.6
Triplo-D	14	3.9	1.0		15	3.3	
Triplo-G	6	5.2	1.5	1.4	3	8.0	
Triplo-H					10	6.5	
Triplo-I	7	6.2	1.3	1.3	7	7.7	

usually about level with the apex of the staminal column (figure 21B); this arrangement probably tends to insure self pollination. As a rule pollen is abundantly produced but exceptionally nearly sterile anthers are found. This type set an abundance of fruit in the field without artificial pollination. The color of the fruit is scarlet. Table 4 shows the results of

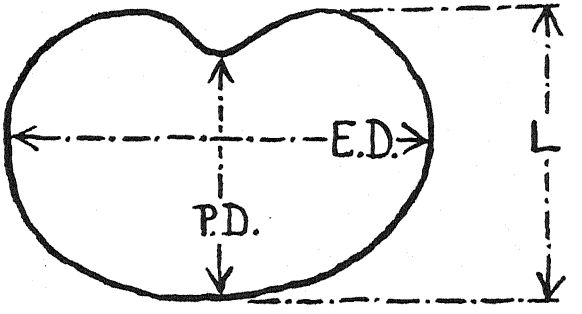


FIGURE 4.—Diagram of fruit. E.D.=equatorial diameter; P.D.=polar diameter; L.=length.

certain measurements of fruits all of which developed in the same season on plants growing in the greenhouse. The meaning of the terms used is shown in figure 4. The values of the ratios of mean equatorial diameter

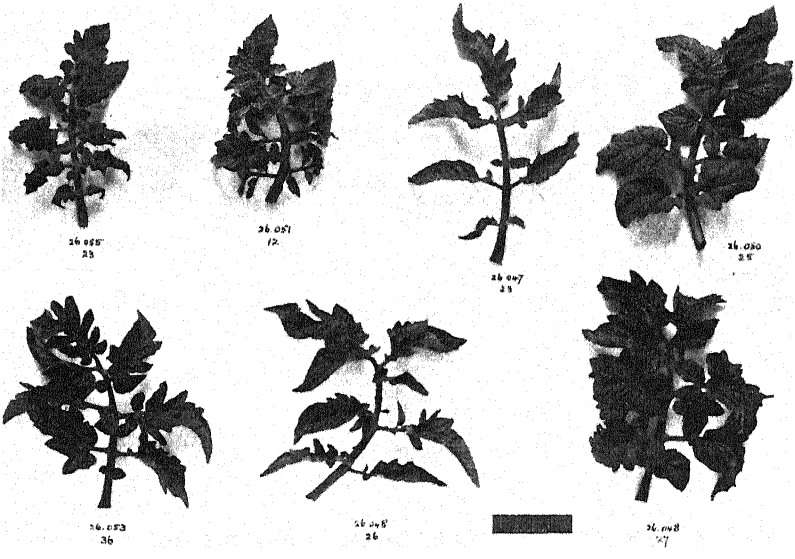


FIGURE 5.—Leaves of diploid and simple-trisomic types of Dwarf Aristocrat (non-hybrid). Triplo-F (26.055.23); triplo-D (26.051.12); triplo-A (26.047.23); triplo-B (26.050.25); triplo-C (26.053.36); diploid (26.048.26); triplo-E (26.048.27).

to length and of length to polar diameter show that the fruit of the diploid is slightly oblate and has shallow polar depressions (figure 25). As a rule it is fairly free from corrugations or longitudinal grooves. The number of loculi or "cells" in the fruit is also shown in table 4, and figure 24D shows a transverse section.

Simple-trisomic types

Triplo-A (figures 5, 7 and 8)

Like the other trisomic types, triplo-A is slower-growing than the diploid and is probably intermediate between the more rapidly growing



FIGURE 6.—Diploid non-hybrid (Dwarf Aristocrat), in field.

trisomic types such as triplo-B and the slower types such as triplo-D. The young foliage tends to be paler than that of the diploid. The leaflets appear to be farther apart, are more acuminate and have fewer deep but more shallow irregular incisions and a more undulate margin. The terminal leaflet is often somewhat twisted and the smaller leaflets or foliolules are much reduced in size. The style is remarkably short; the stigma is about 2 mm below the apex of the staminal cone and often malformed, infolded or without a distinct disc (figure 21A). Under field conditions triplo-A has less pollen and more empty grains than the



FIGURE 7.—Triplo-A non-hybrid (Dwarf Aristocrat), in field.



FIGURE 8.—Triplo-A non-hybrid (Dwarf Aristocrat), in greenhouse.

diploid (table 5). On 88 plants of this type not one fruit set in the field without hand pollination. Only 9 fruits were measured; these were distinctly larger and more oblate and had deeper polar depressions and more cells (figure 24C and table 4) than the diploid fruits.

TABLE 5

Pollen sterility.

	IN FIELD			IN GREENHOUSE		
	Number of grains examined	Bad grains (percent)	Bad grains (percent in total)	Number of grains examined	Bad grains (percent)	Bad grains (percent in total)
Diploid Dwarf Aristocrat	225	12				
"	285	13				
"	231	9	11	177	3	3
Triploid Dwarf Aristocrat				155	75	
"				108	92	87
Triploid Dwarf Champion				84	80	80
Triplo-A, hybrid				202	14	
"	307	66		202	6	
"	334	51	58	139	20	8
Triplo-B, hybrid	272	35				
"	249	33				
"	674	30	31	176	26	26
Triplo-C, non-hybrid	383	64				
"	264	84				
"	957	54				
"	337	70	63	119	17	17
Triplo-D, non-hybrid	252	74	74	135	39	39
Triplo-E, non-hybrid	350	25	25			
Triplo-F, hybrid	274	49				
"	302	26	37			
Triplo-G, non-hybrid	83	27	27			
Triplo-H, non-hybrid	76	27	27			
Triplo-A?, hybrid				295	31	31

A triplo-A hybrid type of standard habit has been described elsewhere (J. W. LESLEY 1926). It has been shown that the extra chromosome present is associated with the locus of the dwarf-standard pair of genes and that the *Ddd* plant, although standard, shows a suggestion of the dwarf character in the greater thickness and rugosity of the leaflets and the shorter style.

Triplo-B (figure 9)

This seems to be one of the more rapidly growing simple-trisomic types. The plant has a characteristic pale olive-green color and metallic cast, especially under field conditions; this difference from the diploid color

may be due to the denser coating of trichomes. The leaflets have a greater tendency to downward or inward rolling of the edges, and the rachis of the leaflets has less tendency to curling than with the diploid (figure 5). The spread of the calyx and corolla is smaller and the stamens shorter than in the diploid. An outstanding characteristic is the length of the style, which is about 1 mm greater than that of the diploid; as a rule the



FIGURE 9.—Triplo-B non-hybrid (Dwarf Aristocrat), in field.

stigma projects considerably beyond the relatively short stamens (figure 22). In the field the quantity of pollen, in proportion to the small size of the stamens, is nearly equal to that of the diploid, and the quality (table 5) was superior to that of triplo-A and among the best of the trisomics. Triplo-B plants often set a few fruits in the field and in the greenhouse without hand pollination.

Triplo-C (figure 10)

This type is intermediate among the simple trisomics in rate of growth, or about equal to triplo-A. The foliage, especially in young plants, is somewhat duller green and the stem is thicker and stiffer and the internodes shorter than in the diploid. The leaflets appear closer together (figure 5) and the edges more uprolled. The peduncles are shorter than



FIGURE 10.—Triplo-C non-hybrid (Dwarf Aristocrat), in field.

those of the diploid. The pollen was scanty and probably inferior in quality to triplo-B in field (table 5) but not in greenhouse plantings. The style appears to be especially variable in length but is on the average shorter than that of the diploid. An interesting characteristic of this type is the greatly increased fasciation of the flower. The number of stamens and other floral organs is much increased; table 4 shows that the

number of loculi in the ovary is more variable but the mean is twice that of the diploid. The interior of some fruits (figure 24A) suggests that of the most highly fasciated varieties such as Trophy. In the field 23 plants of this type set no fruit. Under greenhouse conditions and without artificial pollination, the diploid sets some fruits which are as a rule nearly seedless. Unlike other simple-trisomic types triplo-C sets fruit under these conditions quite as readily as the diploid.

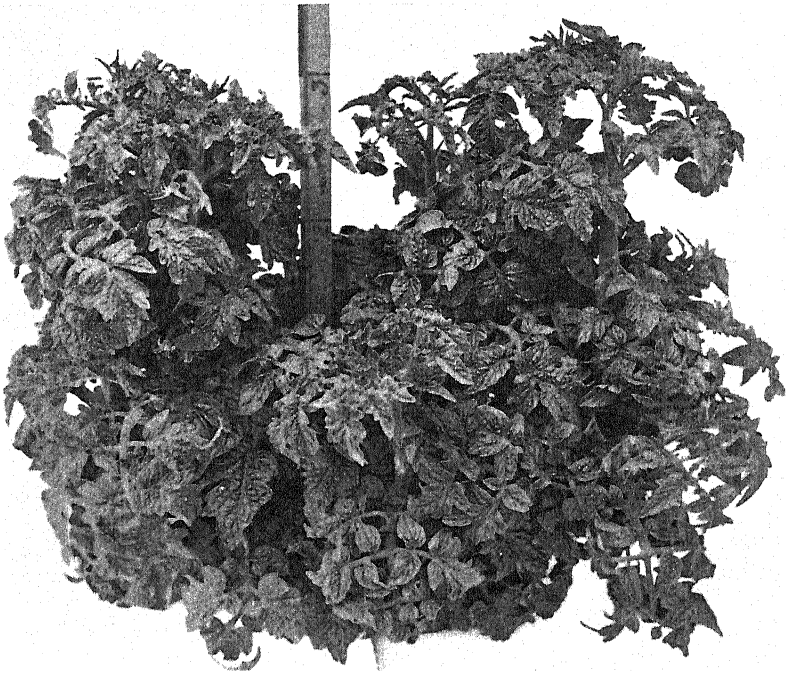


FIGURE 11.—Triplo-D non-hybrid (Dwarf Aristocrat), in field.

Triplo-D (figure 11)

This type is one of the slower-growing simple trisomics and, especially in the field, is far behind the diploid in plant size, previous to fruit setting. The stem is relatively thin, and the leaflets are smaller, narrower and with more deep incisions than in the diploid (figure 5). Most characteristic of this type is the downward (inward) curling of the rachis and of the rachillae, especially of the terminal leaflet. This feature is more marked under field conditions, where the plant presents a compact, almost cushion-like

appearance. The sepals are longer and more recurved (figure 23B) and the stamens more tapering than in the diploid. The quantity of pollen is only slightly less than in the diploid but the quality is probably much inferior (table 5). The flower shows *less* tendency to fasciation than the diploid, and the fruit has fewer loculi, is smaller and distinctly less oblate in shape (table 4 and figures 24E and 25). In the field among 25 plants of



FIGURE 12.—Triplo-E non-hybrid (Dwarf Aristocrat), in field.

this type one set a single fruit without hand pollination. In the greenhouse, after hand pollination, triplo-D set fruit and viable seed nearly as freely as the diploid.

Triplo-E (figure 12)

This is one of the relatively fast-growing trisomics and in general most nearly resembles the diploid. The leaves however are darker green

and have shorter petioles and broader, less acuminate leaflets, giving the leaf a more rounded and less spaced appearance (figure 5). The rachis and rachillae, especially of the terminal leaflet, are more curled and in the field the plant forms a compact, almost pillarlike mass of vegetation. The flower and fruit closely resemble the diploid but the ripe fruit seems



FIGURE 13.—Triplo-F hybrid (Dwarf Aristocrat×Globe F_2 plant), in field.

to be firmer and contains fewer viable seeds. In the field among some 30 plants of this type, 1 set a single fruit without artificial pollination.

Triplo-F (figure 13)

This type is one of the slower-growing simple trisomics. Young plants are much paler green but later the color tends to approach the normal diploid. The leaflets (figure 5) have fewer deep incisions or lobes but more serration. The surface of the leaves has more fine rugosity and is more velvety than that of the diploid. In the field the pollen appears to be of

relatively good quality (table 5) or about equal to triplo-B. Six field-grown plants set no fruit.

Triplo-G (figure 14)

This is one of the more rapidly growing trisomics, like triplo-B and triplo-E. The stems are weaker and the plant is more decumbent in habit than the triplo-C. The leaflets are narrower and comparatively free from



FIGURE 14.—Triplo-G non-hybrid (Dwarf Aristocrat), in greenhouse.

both lobing and serration. Observation of a few specimens indicates that the fruit is more oblate and has a deeper basin (stylar-end depression) than the diploid.

Triplo-H (figure 15)

Only one plant of this type has appeared. It has fewer foliolules and narrower, more acute leaves than the diploid. The leaflets are almost free from lobing and serrations, giving the foliage a very distinctive appearance. The leaf surface unlike that of triplo-I is as rugose as with the diploid. The flower is probably larger than that of the diploid.

Triplo-I (figure 16)

This type resembles the preceding in having fewer foliolules and less lobing or serration than the diploid. It is distinguished from triplo-H



FIGURE 15.—Triplo-H non-hybrid (Dwarf Aristocrat), in greenhouse.

and from the diploid by its less rugose leaves and by paleness of the red flesh color of the interior of the fruit.

Double-trisomic types

Double trisomics are in general even slower-growing than simple trisomics and tend to have less good pollen and less viable seed. The 10



FIGURE 16.—Triplo-I non-hybrid (Dwarf Aristocrat), in greenhouse.



FIGURE 17.—A simple trisomic plant closely resembling triplo-I and perhaps a secondary of it.

non-hybrid double-trisomic plants found belonged to as many different types. This is not surprising since theoretically 9 simple trisomic types may give 36 combination double-trisomic types. Most of the non-hybrid double trisomics were planted in the field. On account of their slow growth and incomplete development, and the small number of plants of each type, adequate descriptions were not obtained. It might be expected that a double trisomic would show a combination of the characteristics of two simple-trisomic types. To some extent, this expectation was fulfilled. Thus one hybrid double trisomic had the curled foliage and small fruit of triplo-D and the pale green color of triplo-F. On selfing both of these types appeared in addition to diploids. One non-hybrid double trisomic was surprisingly like a diploid but had the projecting style and metallic green cast of triplo-B, and like triplo-C it set fruit freely in the greenhouse without pollination. The pollen was very scanty and to obtain progeny it was necessary to backcross to the diploid. The fruits contained but little more seed than the triploid. Among the progeny were triplo-B and triplo-C. A third double-trisomic type clearly suggested

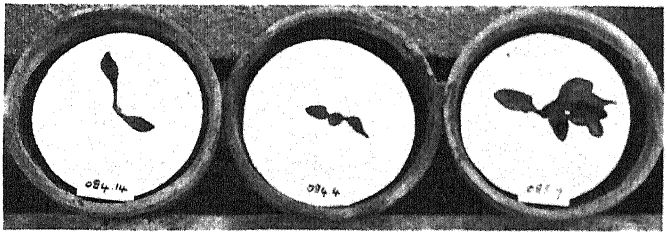


FIGURE 18.—Non-hybrid (Dwarf Aristocrat) "runts"; plants at right and center contained 26 chromosomes in root tips.

triplo-D by its curled foliage and calyx. In other double trisomics the characteristics of simple-trisomic types were not clearly recognized, in some cases perhaps, owing to blending and neutralization of the effects of the extra chromosomes.

A few 26-chromosome plants which originated in the triploid × diploid F_1 and were therefore probably double trisomics, were termed "runts" on account of their imperfect development. The shoot failed to form a bud and consisted of a pair of cotyledons and usually one first leaf which was either tube-like or more normal (figure 18). Owing to the incomplete development and lack of distinctive characters, it is not certain whether they belonged to more than one genotype.

Triple-trisomic types

Two 27-chromosome types were found which, from their origin, were presumably triple trisomics. Although extremely slow-growing they were not "runts" but were perfect in development. Two plants of the remarkable type shown in figure 20 have occurred in the non-hybrid F_1. This type had almost entire leaves like triplo-H, an abnormal stigma like triplo-A and a fasciated ovary like triplo-C; the anthers contained extremely little pollen. The other type (figure 19) was pale green like triplo-F, and curled foliage like triplo-D and had almost entire leaflets like triplo H.

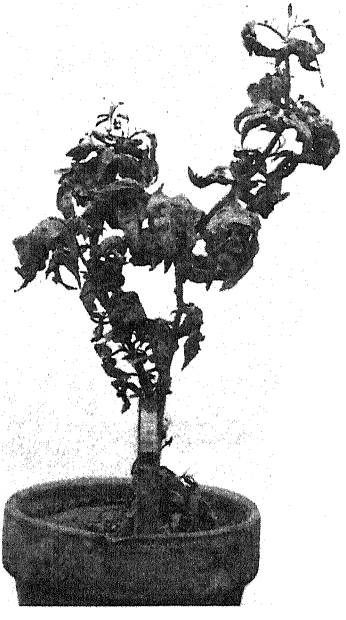


FIGURE 19.—Non-hybrid (Dwarf Aristocrat) 27-chromosome plant with some characteristics suggestive of triplo-D, triplo-F, and triplo-H.



FIGURE 20.—Non-hybrid (Dwarf Aristocrat) 27-chromosome plant with some characteristics suggestive of triplo-A, triplo-C, and triplo-H.

Discussion of trisomic types

According to BLAKESLEE and BELLING (1924), in Datura 11 or perhaps 12 different primary simple-trisomic types have been identified, each containing 1 extra chromosome of the normal haploid set of 12. DE VRIES and BOEDIJN (1924) report that the Oenothera mutants and the progeny

of *semigigas*-forms of *O. Lamarckiana* and *O. biennis* having 15 chromosomes, fall into 7 groups corresponding to the haploid number of chromosomes in *O. Lamarckiana*. Accordingly if all of the 12 chromosomes of the haploid set in tomato are different in their effect, 12 primary simple-

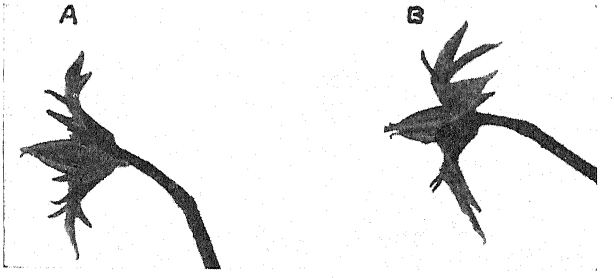


FIGURE 21.—Styles of non-hybrids (Dwarf Aristocrat). A, triplo-A, short; B, diploid, normal.



FIGURE 22.—Style of triplo-B non-hybrid (Dwarf Aristocrat), long.

trisomic types may be expected to occur in the triploid×diploid F_1. Hitherto 9 such types, each with a single apparently whole extra chromosome, have been found. In view of the unequal frequency with which the corresponding types occurred in Datura and of the relatively small number of tomato plants studied, there is reason to think that more simple-trisomic types will be found.

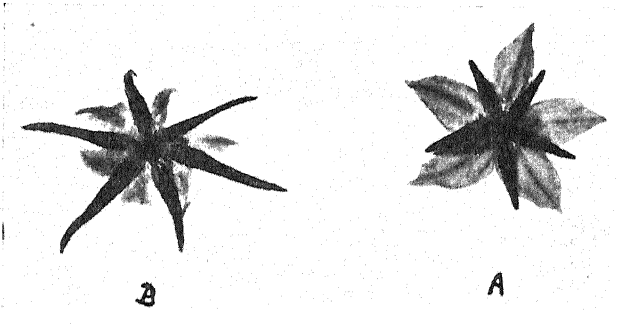


FIGURE 23.—Sepals of non-hybrids (Dwarf Aristocrat). A, diploid, normal; B, triplo-D, long.

For the present, it is preferable to regard these 9 types as homologous with primary mutants of Datura because they originated in the triploid ×diploid F_1, the number of simple-trisomic types found is less than the haploid number of chromosomes and the data (table 6) do not justify the conclusion that any of them is rare. Moreover the 9 types are quite distinct in character and only 1 of the 6 types tested gave trisomic ratios

for dwarf. As will be noted later, 2 of them have occurred as mutants in the progeny of other trisomics but in each case the mutant type showed no special resemblance to the parent and belonged to another type pre-

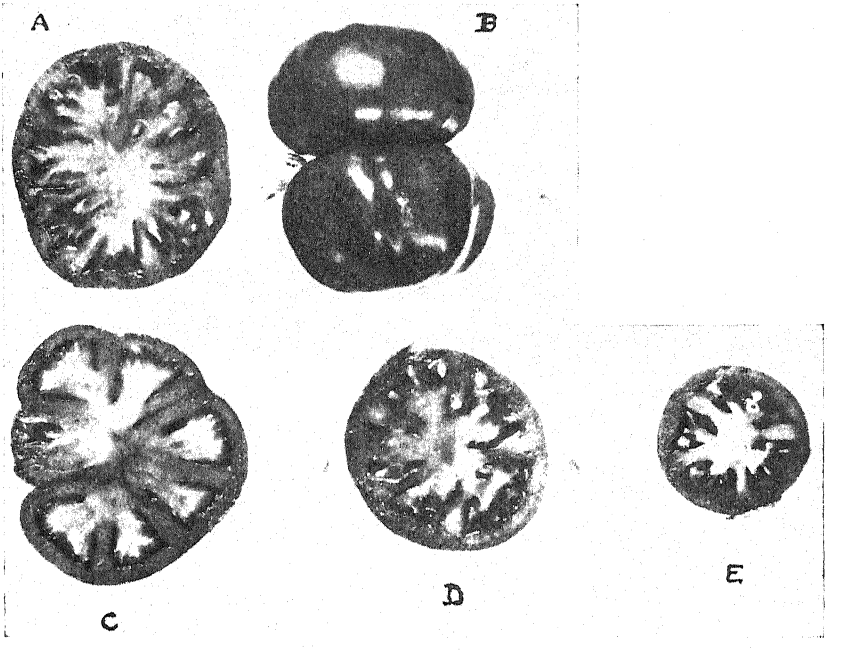


FIGURE 24.—Fruits of non-hybrids (Dwarf Aristocrat). B, triplo-C; transverse sections; A, triplo-C; C, triplo-A; D, diploid; E, triplo-D.

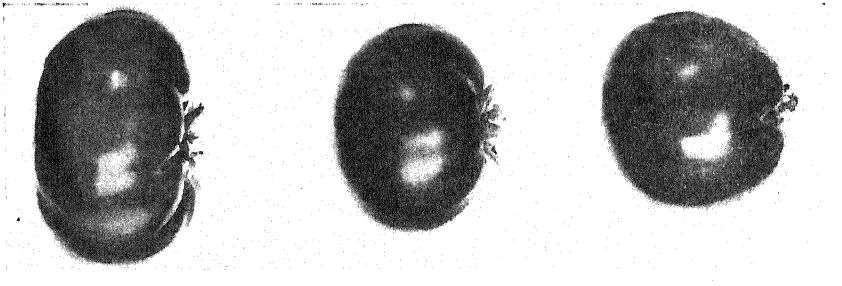


FIGURE 25.—Fruits of non-hybrids (Dwarf Aristocrat). Triplo-G (left); diploid (centre); triplo-D (right).

viously recognized in the F_1 from triploid $\times$ diploid. Moreover there is some cytological evidence to suggest that these mutations resulted from

lagging of members of a normal chromosome pair. On the other hand one plant (figure 17) in the non-hybrid F_1, containing 1 apparently whole extra chromosome, although distinct, resembled triplo-I very closely. Whether this represents another primary type or whether it is a secondary or merely a result of gene mutation cannot as yet be stated. That chromosome fragmentation occurs is shown by the discovery by M. M. LESLEY (unpublished data) of a plant in the non-hybrid F_1 containing 1 whole extra chromosome and an additional fragment, and of other fragment-containing plants in the progeny of trisomics.

It is concluded that the characteristics of each of the 9 simple-trisomic types of tomato are due to the presence of a whole extra chromosome and it is inferred that at least 9 of the 12 chromosomes are different in effect. The fact that no simple-trisomic type has been found which is identical with the diploid, is consistent with the assumption that all of the 12 chromosomes are different.

In haplo-IV and in triplo-IV *Drosophila melanogaster*, BRIDGES (1921) observed a change in the grade of mutant characters due to the IV-chromosome genes, demonstrating clearly the relation between these characters and this particular chromosome. But he also found a change in the grade of mutant characters, the major genes for which were not in the IV-chromosome. This indicated the presence in the IV-chromosome of genes modifying these characters. Similarly in Datura, a single gene difference determines purple and white flower color but BLAKESLEE (1921a) found that in a homozygous purple race, the intensity of color differed widely in the trisomic mutants. Thus Glossy was darker and Cocklebur lighter than the normal. Since neither of these types proved to contain the extra chromosome with the major gene for purple color, the differences indicate the presence of modifying genes in different chromosomes. BRIDGES (1922) has advanced a hypothesis which appears to harmonize admirably with the known facts relating to the distribution and number of major and modifying genes. According to that author, a change in the grade of a character occurs if the extra chromosome present is unbalanced for the genes affecting that character. Now an outstanding feature of the trisomic types of tomato is that many of their distinctive characteristics strongly suggest those which are known to depend on gene differences. Thus the almost entire leaflets of the triplo-H suggest the potato-leaf character, which is a simple recessive to cut leaf. The pale green color of triplo-F is suggestive of the "yellow" foliage character, which according to PRICE and DRINKARD (1908) is a simple recessive to "green" in certain varietal crosses. Differences in number of loculi in the fruit similar to

those seen in triplo-D and triplo-C have been shown by PRICE and DRINKARD (1908) and by WARREN (1924) to depend on gene differences. Compared with the diploid, fruit size is smaller in triplo-D and probably larger in triplo-A. According to LINDSTROM (1926), the evidence clearly indicates that hereditary factors for fruit size and fruit color depend on the same chromosomal mechanism.

Just such a similarity between the effects due to gene mutations and to the presence of extra chromosomes is to be expected according to BRIDGES' hypothesis of gene balance. Moreover, in tomato, a change in the grade of a given characteristic commonly occurs in more than one trisomic type, and in some cases the change is in opposite directions. For example triplo-H tends to have leaflets more nearly entire and triplo-D more dissected than the diploid. Triplo-F is paler green, and triplo-E darker green than the diploid. Triplo-D has fewer loculi in the fruit and triplo-C more than the diploid. This is in accordance with the known fact that number of loculi is not dependent on a single gene pair. Probably this is also true of foliage color and shape of leaflets. Where other evidence suggests that the number of genes influencing a characteristic is relatively small, it is simpler to suppose that the change of grade is due to the presence in the extra chromosome of a single gene influencing the grade of that character. In the case of fruit size, however, which undoubtedly depends on a large number of genes, BRIDGES' conception of gene balance is especially appropriate. Thus the small fruit size of triplo-D may well be due to an excess of modifiers in the minus direction in the internal gene balance of the D-chromosome.

Little is known of the genetic basis of growth rate but it is probably not genetically simple. Since trisomic types differ in rate of growth, it appears that the internal balance of genes affecting growth differs in different chromosomes. Thus the fact that triplo-B is nearer to the diploid in growth rate than triplo-D suggests that the internal balance of genes affecting growth is nearer to the normal (diploid) in the B-chromosome than in the D-chromosome. It seems to be a general rule, to which the tomato conforms, that *no* trisomic type is quicker-growing than the diploid, as if unbalance of chromosome number were never associated with acceleration of growth. In terms of + and − modifiers there is not an excess of plus modifiers in any of the chromosomes. Hence if genes affecting growth are involved, they cannot be adequately classified merely as + and − modifiers but must have more complex relations, perhaps of a complementary nature.

Attention has already been called to the very abnormal development of certain plants, probably double trisomics, which were termed "runts," and to the perfect development of other plants, probably triple trisomics. Clearly these facts cannot be explained on the basis of unbalance in chromosome number. According to BRIDGES' theory of gene balance, however, 2 extra chromosomes with a gene unbalance of the same kind would cause a greater disturbance than 3 extra chromosomes which tend to neutralize one another.

That some attributes of trisomics differ from any gene mutation known in tomato may mean that such mutations have not occurred or have not been preserved. It is possible that some effects due to extra chromosomes, such as curling of foliage and extreme imperfection of development, may not be attainable by any combination of genes in a diploid plant.

FREQUENCY DISTRIBUTION OF OCCURRENCE OF TRISOMIC TYPES

The frequency distribution of the simple trisomic types is shown in table 6. The data do not suggest the great predominance of any one type, and and the absence of three expected types is not surprising considering the small number of plants. Triplo-D, one of the least vigorous types, occurred most frequently, and triplo-H, which is probably one of the more vigorous, occurred least frequently. The chromosome number was not determined in every individual but in all determined cases without exception the same plant type was found to have the same number of chromosomes.

TABLE 6

Frequency of occurrence of simple-trisomic types in triploid×diploid F_1.

	TRIPLO-									TOTAL NUMBER OF F_1 PLANTS
	A	B	C	D	E	F	G	H	I	
Frequencies observed	5	4	5	6	4	3	5	1	3	91

The far more complete data of BLAKESLEE (1924) show that in the progeny of triploid Datura, the simple-trisomic types Echinus and Rolled occur less commonly than Globe and Buckling. In Oenothera DE VRIES and BOEDIJN (1924) found that *cana* occurred more often than *lata* among the 15-chromosome progeny of semigigas (triploid) ×*velutina* (diploid). As already noted the 10 double- and 2 triple-trisomic tomato plants belonged to as many distinct types.

TRANSMISSION OF EXTRA CHROMOSOMES

If the extra chromosome of a simple trisomic is distributed to half of the gametes in meiosis, with equal viability of gametes and zygotes and

with random mating, a simple-trisomic plant, if fertile, on selfing would give 25 percent simple-tetrasomic, 50 percent simple-trisomic and 25 percent diploid progeny; on backcrossing to a diploid, 50 percent simple trisomics and 50 percent diploids would result. That this expectation was not fulfilled is shown by the data from selfing and backcrossing six of the trisomic types shown in table 7.

Several reasons may be suggested for the failure of trisomic plants to transmit the extra chromosomes through the pollen to the full extent. In the pollen mother cells of a simple trisomic the frequent lagging of the unpaired chromosome and the presence of microcytes show that less than

TABLE 7

Transmission of extra chromosomes by trisomic parents.

	POPULATION NUMBER	NUMBER OF PLANTS	GERMINATION PERCENT	PERCENT TRIPLO-					
				A	B	C	D	E	F
Triplo-A hybrid selfed	25.063	47		23					
Triplo-A hybrid×diploid	25.065	88		24				1	
Do.	26.046	72	80	25					
Triplo-A hybrid×Dwarf Champion diploid	25.064	89		18					
Do.	26.047	67	81	33					
Diploid×triplo-A hybrid	25.062	43	80						
Triplo-B hybrid×diploid	26.050	83	93		27				
Diploid×triplo-B hybrid	26.049	90	100		23	1			
Triplo-B hybrid selfed	26.059	92	96		37				
Triplo-C non-hybrid selfed	26.052	61	67			7			
Triplo-C hybrid selfed	25.075	31				26			
Do.	26.056	54	75			20			
Diploid×triplo-C non-hybrid	26.053	52	84			10			
Triplo-D non-hybrid selfed	26.051	64	73				29		
Triplo-E non-hybrid selfed	25.074	33							
Do.	26.048	70	89					16	
Triplo-E hybrid selfed	25.072	53						21	
Do.	26.057	24	89					17	
Triplo-DF hybrid selfed	26.055	34	57				9		24
Triplo-C?×diploid	25.073				18				

half the microspores of the tetrads contain the extra chromosome. But microcyte formation alone does not account for the deficiency in pollen transmission. For triplo-A, which failed entirely to transmit the extra chromosome through the pollen (table 7), had only 4.5 percent of tetrads containing microcytes (table 3), and triplo-B, which gave 23 percent pollen

transmission, had a *greater* proportion of tetrads containing microcytes than triplo-A. The pollen sterility of all trisomic types probably contributes to the low pollen transmission. Since the unbalance in chromosome number of 13-chromosome pollen is as great as that of a simple-tetrasomic plant, the proportion of inviable grains is probably greater among 13-chromosome than among 12-chromosome grains. Triplo-B had fewer "bad" pollen grains than triplo-A or triplo-C and correspondingly transmitted the extra chromosome more readily through the pollen. Triplo-C had less "bad" pollen than triplo-A and higher pollen transmission. Another possible cause of defective pollen transmission is competition between the pollen tubes. Thus BUCHHOLZ and BLAKESLEE (1922) found that, in the Globe mutant of Datura, tubes from pollen with the extra chromosome are slower-growing than those of normal pollen. Consequently the application of an excess of pollen may lead to selective fertilization.

While spore sterility may thus account for defective pollen transmission, it probably does not account for defective transmission through the eggs. BUCHHOLZ and BLAKESLEE (1922) found a larger proportion of aborted ovules from Globe×normal than from normal×normal. Some of these ovules apparently were fertilized and had suffered in competition for food with diploids. In tomato the relatively small number of viable seeds in the fruit of triploids and of trisomic types suggests that the small inviable seeds are those with extra chromosomes. Such zygotic sterility may well account for the deficiency of ovule transmission.

The frequency of transmission of the extra chromosome varied considerably in progenies of similar origin. Thus 26.048 (table 7), the progeny of triplo-E selfed, contained 16 percent, but 25.074, a small population from different seed of the same parentage, contained no trisomic plants. It should be mentioned that these 2 progenies were grown under somewhat different cultural conditions. Again 26.052, from non-hybrid triplo-C selfed, contained 7 percent trisomics, but 26.056 and 25.075, from hybrid triplo-C selfed and from the same seed sample, contained respectively 20 percent and 26 percent triplo-C. These data suggest that the hybrid triplo-C parent transmits the extra chromosome somewhat more readily than the non-hybrid. This may be due in part to the superior germination of the hybrid seed (26.056) compared with the non-hybrid (26.052). According to FROST (1919) it is probable that with the mutant forms of Matthiola, higher frequency of transmission of the extra chromosome is associated with higher percentage of seed germination within the same non-hybrid race.

The frequency of transmission seems to differ significantly in different trisomic types. In hybrids, it ranged from 37 percent from selfing in triplo-B to 20 percent in triplo-C and in non-hybrids from 29 percent in triplo-D to 7 percent in triplo-C. In Matthiola, FROST and MANN (1924) report that in selfing 4 different mutant types, the frequency of transmission by non-hybrids has varied from 25 to 50 percent; later results (FROST, unpublished data) suggest that F_1 hybrid forms tend to give a higher frequency than non-hybrid forms of the same mutant types.

In Datura, the frequency of transmission of the extra chromosome was found to be much lower through the pollen than through the eggs. Thus BLAKESLEE (1921b) reports that the Globe mutant gave about 25 percent transmission through the eggs but only about 3 percent through the pollen. Again CLAUSEN and GOODSPEED (1924) found 35.5 percent of ovule transmission but only 3.4 percent of pollen transmission of the trisomic form "Enlarged" of *Nicotiana tabacum*. In Matthiola on the other hand, FROST and MANN (1924) report that the chromosome or chromosome fragment present in the mutant Slender has been transmitted to nearly 20 percent of the progeny through the pollen. In tomato there seems to be little or no transmission of the extra chromosome through the pollen of triplo-A. For, a small population from diploid × triplo-A contained no trisomic plants and the percentage of trisomics in the progeny of triplo-A hybrid selfed was practically the same as that of triplo-A hybrid × diploids. On the other hand triplo-C transmitted the extra chromosome through the pollen to 10 percent of its progeny. A striking contrast with the results from Datura and Nicotiana was found in the case of triplo-B. Diploid × triplo-B hybrid (26.049, table 7) gave 23 percent triplo-B. The relatively little pollen sterility compared with other simple trisomic types of tomato and the relatively high seed germination in 26.049 are both likely to favour high frequency of transmission. When used as seed parent the same hybrid trisomic gave 27 percent triplo-B or only slightly more than when used as pollen parent. When selfed this plant gave 37 percent triplo-B, which agrees fairly closely with the expectation assuming independence of the processes of transmission through eggs and pollen.

Defective transmission of the extra chromosome, especially through the pollen accounts in part for the scarcity of tetrasomics. Furthermore tetrasomic zygotes are likely to be even less vigorous than trisomics and less able to survive competition with more balanced zygotes. No tetrasomic plants have been found in the progeny from selfing of triplo-A, triplo-C or triplo-E. From the high percentage of pollen transmission

in triplo-B, tetrasomics might be expected in its progeny from selfing. Two remarkably slow-growing plants originated in that way. One of these failed to develop a normal shoot but several adventitious buds developed on the leaves and the flowers resembled triplo-B. Several fruits set from self-pollination. The other plant was normal in development but also far slower-growing than its trisomic sibs. Similarly in the progeny of triplo-D selfed, a single "runt" appeared. The chromosome number of these plants has not yet been determined but they are most probably tetrasomics.

MUTATIONS

No chromosomal mutations other than triploids have been observed by the writer in commercial plantings or in the progeny of plants which were known to be diploid. Two mutant trisomics have occurred, however, in the progeny of trisomic plants. One population (25.065, table 7) from triplo-A hybrid×diploid Dwarf Aristocrat contained 63 diploid, 24 triploid-A and in addition 1 triplo-E plant which must be considered as a mutant. A single mutant plant of triplo-C occurred among the progeny of diploid Dwarf Aristocrat×triplo-B hybrid (26.049, table 7). In simple trisomics the occasional presence of supernumerary laggards in the pollen mother cells points to irregular behavior of a normal pair of chromosomes and not merely of the extra chromosome. In diploids the occurrence of microcytes in the tetrads also suggests irregularities of chromosome distribution. As these irregularities seem to be more frequent in trisomics than in diploids, on the whole it is probable that the mutants resulted from a disturbance of maturation division in the trisomic parents. Since trisomic mutants tend to be unfruitful, some of the "he" plants or "fruitless hybrids" of the tomato grower may be of this nature. Since, however, these mutants have not been observed in the progeny of diploids and are likely to be crowded out in the seedbed probably the "he" plants are usually either triploids or the result of other causes such as mosaic disease.

TRISOMIC INHERITANCE AND LINKAGE

A modification of the Mendelian ratios should occur on breeding from a trisomic plant which is heterozygous for genes whose loci are in the extra chromosomes present. BLAKESLEE and FARNHAM (1923) have shown that the Poinsettia mutant of Datura gives trisomic ratios for flower color, and they conclude that the extra chromosome present contains the locus of the allelomorphic genes which determine purple or white flowers.

Trisomic inheritance of the dwarf character in the tomato has been reported by the present writer (1926). The A-chromosome was found to contain the locus of the genes which determine standard and dwarf. As table 8 shows, 5 other simple-trisomic types heterozygous for the standard-dwarf pair of genes have given apparent disomic ratios for dwarf and standard in accordance with expectation. This is also true of the progeny of C86-8 which belongs to a simple-trisomic type not identified.

Triplo-B, triplo-C and triplo-E hybrids and C86–8, all heterozygous for the pair of genes for cut-leaf and potato-leaf, gave disomic ratios for this pair of characters.

The non-yellow skin color of the fruit is due to the recessive member of a single pair of genes *Yy*. When flesh color is red, *yy* plants have crimson and *Yy* or *YY* plants scarlet fruit. Triplo-A plants gave approximately disomic ratios for skin color, which is in accordance with expectation, since dwarf and non-yellow skin depend on genes which are not linked. The data from other simple- or double-trisomic plants heterozygous for skin-color genes indicate that the locus of this pair of allelomorphs is probably not in the B, C,D, E or F chromosomes, since the ratios among the diploid progeny appear nearer to 3:1 than to the 8:1 ratio which would occur in trisomic inheritance.

A study of the combination of characteristics present in the simple-trisomic types may throw light on the distribution of the genes in the different chromosomes. The simplest case is that in which certain characteristics are peculiar to one only of the simple-trisomic types. For those characteristics, if due to genes, must depend either on a single gene or on linked genes present in that particular chromosome. From the preceding account of the trisomic types, however, it is evident that a change in the grade of a characteristic is usually not confined to one trisomic type. In tomato the I-chromosome probably contains genes which modify flesh color of fruit. Since triplo-I has nearly entire leaves, it may be inferred that, in this case, flesh color of fruit and leaf shape depend on the same gene or on genes which are linked. Again the characteristics of triplo-D suggest that certain genes for size and number of loculi in the fruit are linked. From breeding and correlation studies LINDSTROM (1926) has found evidence of linkage between the genes for dwarf habit and fruit size. Since the A-chromosome contains the locus of the gene for dwarf, triplo-A should accordingly differ from the diploid in size of fruit. The few fruits observed seemed to be distinctly larger than the diploid but more data are required to establish this. The relatively small size of seedless fruits and of the few-seeded fruits regularly produced by the

TABLE 8[6]

Inheritance of habit, leaf form and skin color of fruit.

(D = standard, d = dwarf habit; C = cut, c = potato leaf; Y = yellow, y = non-yellow skin of fruit.)

	HABIT AND LEAF FORM								SKIN COLOR OF FRUIT				THEORETICAL MONOHYBRID RATIOS				
													Disomic inheritance	Trisomic inheritance			
	Diploid progeny				Trisomic progeny				Diploid progeny		Trisomic progeny		Diploid and trisomic progeny	Diploid progeny		Trismoic progeny	
	DC	*Dc*	*dC*	*dc*	*DC*	*Dc*	*dC*	*dc*	*Y*	*y*	*Y*	*y*	*D*:*d*, *C*:*c* or *Y*:*y*	*D*:*d*	*C*:*c Y*:*y*	*D*:*d*	*C*:*c Y*:*y*
Triplo-A hybrid selfed	60		56		8		3		17	11			3:1	5:4	8:1	7:2	all dominants
Diploid × triplo-A hybrid	13		30						18	18			1:1	1:2	2:1	2:1	do
Triplo-B hybrid selfed	54	7	17	7	15	9	6	5	54	11	5	1	3:1	5:4	8:1	7:2	do
Triplo-B hybrid × diploid	37		24		15		7						1:1	1:2	2:1	2:1	do
Diploid × triplo-B hybrid	37		32		6		15						1:1	1:2	2:1	2:1	do
Triplo-C hybrid selfed	35	19	12	9	7	3	4		40	14			3:1	5:4	8:1	7:2	do
Triplo-E hybrid selfed	41	11	14	8	10	2	1		29	11			3:1	5:4	8:1	7:2	do
Triplo-DF hybrid selfed	17		5		11		1		14	8			3:1	5:4	8:1	7:2	do
Simple trisomic hybrids of unknown type: C86-2 selfed	38	9	9	5	5	2			26	13			3:1	5:4	8:1	7:2	do
C86-7 selfed	23	13	7	4					19	7			3:1	5:4	8:1	7:2	do
C86-8 selfed	33	9	7	7	5	2	2	2	42	12			3:1	5:4	8:1	7:2	do

[6] The writer is indebted to MR. C. W. HANEY, Division of Genetics, UNIVERSITY OF CALIFORNIA, for part of the observations contained in this table.

triploid, point to some degree of correlation between fruit size and seed production in tomato. If so, comparison of fruit size between trisomic types with few seeds such as non-hybrid triplo-A and the diploid is more difficult. CRANE (1915) has found some evidence of linkage between the genes for simple inflorescence and short, that is, oblate, fruit. MACARTHUR (1926) has shown that simple inflorescence and dwarf are linked. Hence triplo-A, which contains the locus of dwarf, should also contain factors modifying length of fruit. The scanty data available (table 4) suggest that this is in fact the case, since in triplo-A, ED/L is 1.44 as compared with 1.25 in the diploid. LINDSTROM (1926) has found good evidence of linkage between *Yy*, the genes for skin color of fruit, and certain major factors determining size of fruit. Hence the simple-trisomic type with the extra chromosome containing the locus of *Yy* should also contain major genes affecting fruit size. As noted above a type giving trisomic ratios for skin color of fruit has not yet been found.

CONCLUSION

The present paper represents only a beginning of the analysis of the progeny of triploid tomatoes. In particular much more light can be thrown on the composition of the individual chromosomes by a more detailed study of the types and by hybridization. The occurrence of chromosome fragmentation is of special interest and may also throw light on the distribution of the genes and on the problems of size and number of chromosomes.

The foregoing data appear to be wholly consistent with the chromosome theory of heredity. In infectious disease certain conditions, defined by ROBERT KOCH in 1882, are required to prove the causal nature of the infecting organism. A rather close analogy may be drawn between such a pathogenic organism and a chromosome. Extra chromosomes occurred invariably and exclusively in plants with certain variant characters, thus conforming to two of the famous canons. As in the case of obligate parasites the chromosomes have not been grown in culture apart from the host as the third canon requires, but they are transmitted to the progeny and reproduce identical symptoms. The simplest conclusion is that the relation between the extra chromosome and the characteristics of a trisomic plant is causal. To hypothecate an unknown underlying cause of which an extra chromosome is merely a symptom would seem to be gratuitous.

The occurrence of trisomic ratios in the progeny of a hybrid trisomic tomato plant is a further confirmation of the association between chromo-

some and gene. Evidently in the tomato unbalance in terms of chromosome number is an important factor influencing growth and viability. The effects of extra chromosomes on development are such as might result from differences in internal gene balance in different chromosomes, as defined by Bridges, and at present the most reasonable hypothesis is that certain of the effects associated with the presence of extra chromosomes are due to a change in the balance of the genes.

The visible effects caused by a single whole extra chromosome are in some cases small compared with those due to single gene differences. For example the effect of the E-chromosome in triplo-E is less striking than that of the gene causing potato leaf. On the other hand the effect of the D-chromosome in triplo-D is probably greater than that of certain genes. Indeed from its appearance, triplo-D would readily pass as a distinct variety, but owing to its partial sterility and genetic inconstancy, so characteristic of trisomic types, it can scarcely be of importance in the evolution of new varieties.

SUMMARY

1. Triploid plants have been found in 3 varieties of tomato. They probably originated from the fusion of diploid and haploid gametes.

2. In the F_1 from triploid×diploid, chromosome number did not have a binomial distribution but ranged from 24, the diploid somatic number, to 27, with the mode at 25 chromosomes. Degree of unbalance in chromosome number is therefore an important factor influencing viability.

3. The smaller seeds from triploid×diploid gave more 26- and 27-chromosome plants than the larger seeds.

4. From diploid×triploid, only 2 plants were obtained, both of which were diploid.

5. In meiosis in the pollen mother cells of a simple trisomic, the unpaired chromosome is not constant in its behavior. At diakinesis it may either form a trisome or lie separate. It frequently lags in the first and second anaphase. In the heterotype division it may undergo either assortment or division, more often the former. Correspondingly in the homotype division either division or assortment takes place, and probably assortment requires more time than completion of the process of division.

6. The proportion of tetrads containing microcytes varied from 4.5 percent to 7.6 percent in different simple-trisomic types. The presence of micronuclei at interkinesis in the pollen mother cells suggests that microcyte formation sometimes begins at that stage.

7. In general, compared with the diploid, simple-trisomic types are slower-growing and have a greater tendency to pollen sterility and unfruitfulness in the field. These differences are accentuated in double- and triple-trisomic types.

8. From the haploid number of chromosomes, 12 different simple-trisomic types are expected. Of these, 9 have been identified. At least 9 of the 12 chromosomes of the haploid set are therefore different in their effect on development.

9. The 9 simple-trisomic types are "primaries" in the terminology proposed by BLAKESLEE and are due to the presence of a whole extra chromosome of the normal set. Types containing fragments also occur.

10. No variation in chromosome number was found among individuals of the same type, when examined, they invariably proved to have the same number of chromosomes except in the case of tetraploid islands (p. 4).

11. Each of the simple-trisomic types has a distinctive combination of characteristics. In several cases two or more types differ from the diploid in the same characteristic but to a different degree and in different directions.

12. The double- and triple-trisomic types have, in some cases, combinations of the characteristics of simple-trisomic types.

13. Some of the effects of an extra chromosome are due to modifying genes present in it. Where the number of genes affecting a characteristic is large, the effects of an extra chromosome are probably the result of an internal gene balance of the extra chromosome, different from that of the normal chromosome complement.

14. Certain double trisomics are more abnormal in development than certain triple trisomics. Two extra chromosomes with an internal gene balance in the same direction may cause a greater disturbance of development than three extra chromosomes which tend to neutralize one another.

15. Among 35 simple-trisomic plants of 9 different types, no type predominated greatly in number of individuals.

16. The extra chromosome is transmitted to less than the expected proportion of the progeny. The observed proportion from selfing varied with different extra chromosomes. In two simple trisomic types the extra chromosome was transmitted through the pollen almost as frequently as through the eggs. Defective transmission through the pollen is in part due to irregularities in sporogenesis and in part perhaps to pollen sterility and selective fertilization. Defective transmission through the eggs is probably due, in part, to zygote sterility.

17. Tetrasomics probably occur, although rarely, in the progeny of certain simple trisomics.

18. Simple-trisomic types sometimes occur as mutants. In pollen mother cells of trisomics the occasional lagging of members of a normal pair and the occasional presence of microcytes in the tetrads of a diploid suggest how these mutants may arise.

19. Trisomic inheritance of the dwarf character has been found and the simple-trisomic type whose extra chromosome contains the locus of dwarf has been identified.

20. Evidence of linkage between genes may be obtained from a knowledge of the combinations of characteristics occurring in simple-trisomic types, especially when this information is supplemented by results of correlation studies and breeding experiments.

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FIVE AUTOSOMAL MUTANTS IN *DROSOPHILA HYDEI*[1]

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INTRODUCTION

A description of *Drosophila hydei* Sturtevant is to be found in STURTEVANT'S monograph on the North American species of Drosophila (1921). It is of particular interest because of its very close resemblance to *D. repleta* and *D. mulleri* and because it is one of the few very common species of the genus. Sex-linked mutants have been described by CLAUSEN (1923) and SPENCER (1927). An autosomal recessive eye color, scarlet, was found by HYDE (1915), and the same author (1922) has reported another mutant, eyeless, a recessive linked to scarlet with about 25 percent of crossing over. The present paper deals with the origin, description, and linkage relations of five new autosomal recessive mutants, red, orange, triangle, facet, and extra-scutellars. Unfortunately HYDE'S scarlet and eyeless could not be used in linkage studies as they had been lost before the finding of the mutants here described.

RED (*r*), A RECESSIVE EYE COLOR

In a large stock bottle of *D. hydei*, out of a thousand or more flies examined on March 3rd, 1926, several were found with a red eye color. Breeding tests have shown this mutant to be an autosomal recessive of fair viability. The eye color, particularly in the newly emerged fly is a bright red

[1] This work was done at the OHIO STATE LAKE LABORATORY, Put-in-Bay, Ohio and the Biology Laboratory, COLLEGE OF WOOSTER, Wooster, Ohio. I wish to thank Doctors R. C. OSBURN and W. M. BARROWS of OHIO STATE UNIVERSITY for their interest in and encouragement of the work and Doctor R. E. COLLINS of the UNIVERSITY OF CALIFORNIA for furnishing stocks of the white and vermilion mutants of *D. hydei*.

in contrast to the dark dull brown of the wild type, and resembles closely the wild type eye color of *D. melanogaster*. This color darkens considerably with age and approaches more nearly the wild type, but in most cultures can be separated without difficulty even when the flies are old. The description of HYDE'S scarlet indicates that it was somewhat more brilliant than red. The stock in which this mutant arose was derived from a wild stock collected in a fruit cellar in Wooster, Ohio, and this wild stock will be referred to as Wooster stock.

ORANGE (*o*), A RECESSIVE EYE COLOR

On May 10th, 1926, among several hundred flies removed from a large stock bottle of Wooster stock two females were found having an orange eye color, strikingly different from the wild type. These females were each mated to normal-appearing males from the same bottle. One of these cultures gave all wild type while in the other approximately half the flies were orange eyed. These results showed that orange was an autosomal recessive and that the male in the second culture had been heterozygous for the orange gene. The excellent viability and ease of classification of orange make it especially valuable for linkage studies. This eye color is somewhat like peach of *D. melanogaster* and when compared with charts in Ridgway (1912) is between the colors listed by him as Mars Orange and Burnt Sienna. The color darkens with age and there is little or no shadow in the eye.

Preliminary breeding tests showed that red and orange were linked. A double recessive stock was established. This eye color was easily separable from both red and orange and cultures were then made up of red orange males to females with orange in one chromosome and red in the other. The crossover data from these are given in table 1. Of 3089 flies, 207, or 6.7 percent, were crossovers. The data from the reciprocal type of cross in which red and orange entered in the same chromosome are given in table 2. From 1028 flies, 66 were crossovers, giving a percentage of 6.42. On combining the data out of a total of 4117 flies 273 or 6.63 percent were crossovers. The tentative loci of these mutants are therefore placed as follows: red (0.0) and orange (6.6) in the second linkage group. (This is called the second linkage group as HYDE'S two linked autosomals were not available for testing linkage relations to the genes reported here.)

TRIANGLE (*t*), A RECESSIVE WING VEIN MUTANT

On January 14th, 1927, in counting flies from culture 827 of the red mutant five normal males, eight normal females, and five males and eight

females showing a slight variation in wing venation were found. When culture 682 from which 827 had been derived was examined, it was found to contain a few flies with the same variation. This culture was made up on November 13th, 1926, and it is probable that the mutant had arisen in the red stock previous to this date.

The character expression of triangle varies from a short extra cross-vein connecting the costal and second veins near the posterior termination of the latter, to two or three of these short cross-veins or a triangular deltoid area formed by the confluence of the costal and second veins for a short distance near the posterior termination of the latter. The character resembles the triangle and confluent mutants in *D. virilis* described by METZ, MOSES and MASON (1923) but differs in being a recessive. In cultures homozygous for triangle many flies show the character only in one wing and others are entirely normal in appearance. Sometimes only a few flies show the character; this is particularly true if the culture is crowded. For this reason triangle is of little value in linkage studies.

As triangle arose in a red stock it was easy to test the two mutants for linkage. It was found that triangle was linked to red and orange. A red orange triangle stock has been made up and the crossover data thus far secured indicate that triangle crosses over freely with the other two loci. However, its extreme variability in the number of normal overlaps of homozygous triangle flies makes it difficult to determine with any degree of accuracy its position relative to red and orange.

The author has found a recessive mutant in *D. melanogaster* (unpublished) which very often resembles triangle. It is interesting to note that this recessive triangle in *D. melanogaster* lies in the third chromosome linked to scarlet and peach, two eye colors which are rather closely linked and which resemble the closely linked red and orange mutants of *D. hydei*. The linkage of these three similar mutations in the two speces is some indication that they are really parallel mutations.

FACET (*f*), A RECESSIVE EYE TEXTURE MUTANT CAUSING STERILITY IN MALES

In examining flies from culture 969, a stock of the sex-linked mutants Notched-vermilion-bobbed, on February 13th, 1927, one male and two females, not Notched, were found having the hairs on the eyes slightly disarranged and many of the facets rounded up so as to give an appearance of glittering points of light reflected from the surface of the eye. An examination of the parent Notched-vermilion-bobbed stock made up on December 12th, 1926, revealed one such facet female and it is likely that the mutation occurred previous to this in the Notched-vermilion-bobbed

stock. Examination of other Notched-vermilion-bobbed cultures showed the presence of facet in them. The presence of vermilion in the culture probably accounts for the detection of this mutant as the facet character shows up more strikingly against the bright vermilion background. However, the character is readily classified in the presence of any of the mutant eye colors or with the wild type eye color.

TABLE 1

Linkage of Red and Orange. Red Orange $\frac{ro}{ro}$ *males* $\times$ $\frac{rO}{Ro}$ *females.*

CULTURE NUMBER	NON CROSSOVERS				CROSSOVERS				TOTAL
	Red ♂	Red ♀	Orange ♂	Orange ♀	Red Orange ♂	Red Orange ♀	Mahog-any ♂	Mahog-any ♀	
777	17	22	51	67	0	0	3	5	165
803	19	34	57	47	0	2	4	4	167
804	28	20	33	37	1	1	4	4	128
806	44	40	60	41	1	1	5	2	194
807	18	19	31	27	2	1	7	3	108
808	37	19	31	44	1	2	7	4	145
809	11	18	35	32	1	1	3	2	103
857	8	22	13	31	0	0	3	3	80
858	8	4	9	10	0	0	1	2	34
859	8	21	28	35	0	1	2	6	101
860	18	29	24	43	1	0	1	3	119
861	29	38	54	44	1	2	4	7	179
863	23	34	34	35	0	0	7	3	136
864	20	9	20	23	0	1	0	5	78
865	4	2	8	0	1	0	0	0	15
866	24	29	35	37	0	0	4	3	132
867	30	24	12	30	1	1	1	3	102
868	21	24	25	29	0	0	2	3	104
869	30	40	26	43	0	1	4	3	147
870	31	31	45	45	1	2	2	3	160
871	23	24	16	23	1	2	1	1	91
872	26	16	27	21	0	2	3	2	97
873	33	39	34	48	2	1	3	11	171
874	20	18	26	24	0	2	5	1	96
875	29	25	27	18	0	1	4	0	104
876	10	9	14	12	0	0	0	2	47
877	18	21	25	18	0	0	0	4	86
Totals	587	631	800	864	14	24	80	89	3089

Total flies 3089; total crossovers 207; crossover percentage 6.7.

TABLE 2

Linkage of Red and Orange. Red Orange $\frac{ro}{ro}$ *males* $\times$ $\frac{RO}{ro}$ *females.*

CULTURE NUMBER	NON CROSSOVERS				CROSSOVERS				TOTAL
	Mahogany ♂	Mahogany ♀	Red Orange ♂	Red Orange ♀	Red ♂	Red ♀	Orange ♂	Orange ♀	
936	54	55	26	25	2	2	2	4	170
937	31	47	14	30	4	2	2	4	134
938	26	43	39	32	1	0	6	3	150
939	37	39	25	26	2	0	2	2	133
940	36	35	42	29	2	2	4	3	153
941	33	45	33	30	1	4	2	4	152
942	48	38	21	23	4	2	0	0	136
Totals	265	302	200	195	16	12	18	20	1028

Total flies 1028; total crossovers 66; crossover percentage 6.42.

Two crosses of vermilion facet females to vermilion facet males and seven crosses of vermilion orange females to vermilion facet males were made. Although subcultured twice none of these produced any offspring. Facet females were then selected and crossed to vermilion orange males. These crosses produced wild type offspring, showing that facet was recessive and not sex-linked. In the F_2 generation some facet orange flies appeared indicating that facet was not linked to red, orange and triangle. Fully thirty facet males have been tested and all have been entirely sterile. The females, however, are of normal fertility and the viability of facet is excellent. The stock is maintained by selecting in each generation homozygous facet females and crossing to their heterozygous facet brothers.

EXTRA-SCUTELLARS (*e*), A RECESSIVE BRISTLE CHARACTER

On March 14th, 1927, in culture 993, which was the F_2 generation of a cross of the sex-linked vermilion male to a female from a stock received from Doctor A. H. STURTEVANT from Kansas City, Kansas, two vermilion females and one vermilion male were found having small extra-scutellar bristles. These were mated and a large percentage of the flies from this cross had extra-scutellars. Subsequent tests have shown the character to be an autosomal recessive and not linked either to red, orange, and triangle or to facet. The character expression varies from one to three extra-scutellars. The bristles are smaller than the other scutellars and the most frequent position is just back of the anterior scutellars, or in the midline at the posterior edge of the scutellum. Where three bristles

are present one is found in the midline and one on either side. Generally in homozygous cultures some of the flies fail to show the character, and as in the case of triangle the percentage of such normal overlaps is quite variable. For this reason the mutant is of little value other than at present it represents a new linkage group in *D. hydei*.

DISCUSSION AND SUMMARY

METZ and MOSES (1923) have described the chromosome groups of many species of drosophila. According to their figure that of *D. hydei* consists of six pairs of chromosomes of which one pair is very small. The mutations reported in this and other papers fall into four linkage groups. Those in the X chromosome with their approximate loci are Notched (0.0) white (3.8), vermilion (15.5), and bobbed (63). In the first autosome lie red (0.0) orange (6.6), and triangle (locus not accurately determined). Facet lies in the second autosome and extra-scutellars in the third autosome. It is not unlikely that continued investigation of the species will result in the finding of a fifth linkage group, and possibly of a sixth. The finding of four sex-linked mutants similar to sex-linked mutants in *D. melanogaster* and of three linked autosomal mutants, red, orange, and triangle similar to the three third chromosome mutants of *D. melanogaster*, scarlet, peach, and triangle is additional evidence indicating that there is some correlation in the configuration of the chromosome maps of various species of Drosophila.

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INHERITANCE STUDIES IN SOYBEANS. III. SEED-COAT COLOR AND SUMMARY OF ALL OTHER MENDELIAN CHARACTERS THUS FAR REPORTED[1]

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INTRODUCTION

In 1923 the writer began a study of the inheritance of seed-coat colors in the soybean (*Soja max*). From a practical standpoint, the problem is of great interest because seed-coat colors are very important in the selection of pure strains and in the detection of mixtures in commercial varieties. This led to a rather intensive study of the factors concerned in mottling, the results of which are being published in another paper.

The results of previous workers indicated that two varieties may look alike but may behave very differently in crosses. This made it advisable to secure numerous crosses among a large number of varieties in order that the genetic constitutions of different types could be compared. Al-

[1] From the Department of Genetics Paper No. 74 and the Department of Agronomy, WISCONSIN AGRICULTURAL EXPERIMENT STATION. Published with the approval of the Director of the Station.

though the original plans have by no means been realized, over 300 hybrids, representing 35 different combinations, have been produced.

Some of the parental plants used for hybridization were from well known varieties, but others were from selections made by the Agronomy Department of the WISCONSIN AGRICULTURAL EXPERIMENT STATION, and their origin is uncertain. It has been necessary to assign arbitrary numbers to these selections which have not been identified as belonging to any particular variety.

With but few exceptions all factors studied in soybeans have something to do with the color of the seed coat, either directly or through linkage. It seems logical, therefore, to center the discussion on seed-coat colors although a complete list is given of all the genetic factors that have been reported.

In carrying out the problem the writer has been greatly aided by counsel from Professor B. D. LEITH of the Agronomy Department and Professors L. J. COLE and R. A. BRINK of the Genetics Department of the UNIVERSITY OF WISCONSIN. It is a pleasure to acknowledge all the advice and constructive criticisms which have been received.

SEED-COAT COLOR TYPES

NAGAI (1921) and PIPER and MORSE (1923) have given classifications of seed-coat color types for soybeans, but for the purpose of interpreting Mendelian characters the following classification has been found most useful.

I. Self-color type
   1. Black
   2. Brown (may be of different intensities according to the variety and somewhat according to conditions at the time of maturity)
   3. Imperfect black } (Associated with gray pubescence)
   4. Buff }

II. Bicolor type
   1. Black mottling pattern on brown background

III. Eyebrow pattern with green or yellow background
   1. Black
   2. Brown
   3. Imperfect black } (Associated with gray pubescence)
   4. Buff }

IV. Green or yellow seed coat with dark hilum. (Mottling is developed over the remaining portion of the seed coat according to environmental conditions.)

1. Hilum black
2. Hilum brown
3. Hilum imperfect black } (Associated with gray pubescence)
4. Hilum buff }

V. Green or yellow seed coat with light hilum. (Mottling is produced according to environmental conditions but pigments are always potentially present.)
1. Mottled with black
2. Mottled with brown
3. Mottled with imperfect black } (Associated with gray pubescence)
4. Mottled with buff }

PIGMENTS RESPONSIBLE FOR SEED-COAT COLORS

What has been designated as black pigment in the seed coat of the soybean is really a very intense purple and belongs to the general class of anthocyanins. The brown or phlobaphene-like pigments are undoubtedly closely related to quercetin but their exact chemical composition is not known. In the imperfect blacks there is a mixture of the two pigments and even in the self-blacks a considerable amount of brown pigment is masked by the black.

In addition to the pigments mentioned above, which are glucosides and have many properties in common, there is another blackish-brown pigment which behaves very differently. In all probability it belongs to the general class known as "oxidation pigments" because of their connection with the process of oxidation. This is the pigment responsible for the common blackish-brown pod color in black-podded varieties but this same pigment may also find its way into the parenchymatous cells of the seed-coat. Frequently it produces such a smudgy appearance that a classification of a plant in regard to the other pigments is rather uncertain. Unlike the anthocyanins and phlobaphene-like pigments, the production of the oxidation pigment may be greatly increased by injury to the pod and testa.

Another colored condition has been described by MATSUMOTO and TOMOYASU (1925) which they have called "purple speck." The pigment responsible for this abnormal coloration, according to the descriptions, has similar properties to anthocyanins but a fungus (*Cercosporina Kikuchii*) was found to be responsible for its occurrence.

The black and brown pigments, with which the present article is chiefly concerned, are independent of the green and yellow plastid pigments. In self-browns or mottled types not all of the plastid color is concealed,

so it is usually possible to detect the presence of green when it is present but in self-blacks plastid colors are entirely masked.

THE VARIABILITY OF SEED-COAT COLORS

Except in self-colored types there is a great deal of variability in the amount of pigment produced in the seed coat. This has been regarded as objectionable by practical growers, so the subject of mottling has received considerable attention in all the important soybean-growing sections of the United States. Much effort has been spent in selecting for non-mottled strains but all varieties which have been tested in cooperation with the Agronomy Department of the WISCONSIN AGRICULTURAL EXPERIMENT STATION have produced mottling in some degree. Some selections have proved to be much more badly affected than others but mottling seems to be a general phenomenon with all yellow- and green-seeded varieties. Details of this work are being published in another paper.

WOODWORTH and COLE (1924) have given evidence to show that environmental factors may be important in bringing out mottling pigments and their conclusions have been well confirmed during the progress of this study. Varieties which failed to develop any signs of mottling in the greenhouse during winter months were badly mottled in the field.

A rich loam soil has consistently produced much more mottling than a light sandy soil, but the distance of spacing plants has also been found to be very important. Plants grown three feet apart have produced more mottled seed than plants grown closely together, but on certain types of soil the distance of spacing has not been a determining factor. These results are confirmed by HOLLOWELL'S (1924) findings at Iowa.

Although it seems probable that there may be many factors which affect mottling, perhaps the most important is the maturation of the seed. An extreme amount of mottling was found to be associated with plants which remained green after the normal time for the seeds to mature.

Environmental factors have no apparent influence in the case of self-colored varieties, because no difficulty is experienced in getting these varieties to breed true; but there are noticeable differences in intensity of color due to environment. The imperfect-black type is extremely variable in its appearance. Sometimes seeds are nearly black and in other cases some experience is required to distinguish this type from light brown. NAGAI (1924) gives evidence to show that this variability is not independent of inheritance but the present writer has no data on the relative importance of environment and heredity on this particular coloration.

The self-browns and buffs are also variable in regard to the intensity of color and conditions at maturity seem important in this connection.

For a genetic study of seed-coat colors it is usually desirable to have the plants growing in an environment that will produce the maximum amount of pigment. This together with an intimate knowledge of the variability that might be expected due to environment is necessary in order to classify intelligently certain F_2 progenies.

RESULTS OF PREVIOUS INVESTIGATIONS

PIPER and MORSE (1923) found black seed coat dominant to brown. In the particular example cited a single factor was responsible for the difference. NAGAI (1921) also found black to be dominant to brown but he distinguished between different types of blacks and browns. He demonstrated the presence of a factor *R* which produced anthocyanin pigment and a factor *C* which increased the intensity of pigmentation. According to NAGAI'S hypothesis, with *CR* present the seed coat is black; with *cR* it is an imperfect black; with *Cr* it is brown; and with *cr* it is a very light brown or "buff."

In addition to *C* and *R*, NAGAI assumed that another factor *O* must be present in order to explain the inheritance of certain browns. A rich reddish brown was recessive to the common dull brown. Therefore, with *CO* present the pigment was a dull brown, and with *Co*, reddish brown; but the relation between the two factors, *C* and *O*, was not entirely clear.

WOODWORTH (1921) observed that two factors, *B* and *H*, were necessary for the production of black pigment in the hilum, but *H* was found to be completely linked to *T*, the factor for tawny pubescence. Plants homozygous for *ht* always produced gray pubescence and a seed coat with a brown hilum. In Part II of this series another factor, *E*, was added to this linkage group. This factor *E* is a partially dominant factor for early maturity but since the expression was not always clear-cut it was only through the linkage relation that its effect could be attributed to a single factor. There was evidently a small percentage of crossing over but this could not be accurately measured.

NAGAI and SAITO (1923) found self-blacks or self-browns recessive to yellow or green and they have described three restriction factors. According to these writers, the factor *K* inhibits the production of pigments forming a "patched" or "eyebrow" pattern; *I* inhibits nearly completely the formation of chromogenic substance; and *H* inhibits it entirely. The recessives of these factors have no effect.

NAGAI and SAITO also found a single factor *M* responsible for the production of a black mottling pattern upon a brown background. They assumed that *M* is linked to the factor *P* which inhibits the formation of pubescence, giving a crossover value of approximately 18 percent. In Part II of this series the writer also reports a linkage between *P* and R_1, a factor for anthocyanin pigment. This linkage appears to be nearly complete with only occasional crossovers. Mention is also made of the factor p_2 for glabrousness reported by STEWART and WENTZ (1926) which was recessive to P_2, a factor for pubescence. Thus we have a character (glabrousness) which is dominant in one case and recessive in another case; apparently due to different factors.

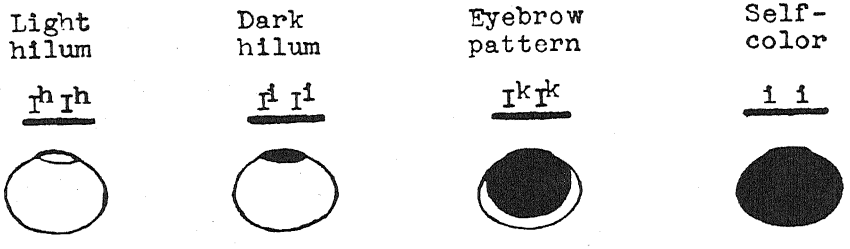
TAKAHASHI and FUKUYAMA (1919) have given results obtained in F_2 from five different crosses but no attempt was made to give an interpretation, due to the small amount of data available. Their results at least indicate that varieties are green or yellow because of the presence of factors that inhibit pigment formation. More blacks than browns were secured in the F_2's but the data do not warrant conclusions in regard to the factorial relationships.

NAGAI (1921) found that black and brown pigments are formed regardless of whether the plastid pigments in the seed coat are green or yellow. Green, however, was dominant over yellow. WOODWORTH (1921) obtained identical results but found that *V*, the factor used for green seed coat, was linked with *D*, a factor for the production of yellow cotyledons, giving about 13 percent crossing over; but *V* was assumed to be independent of another factor *I* which is a duplicate of *D* and also produced yellow cotyledons. These findings have been well confirmed by the writer and the results are briefly presented in Part I of this series.

TERAO (1918) found a single factor responsible for green seed coat color when the maternal parent was yellow, but when the maternal parent was green there was no segregation, the F_1's and F_2's being the same color as the female parent. PIPER and MORSE (1923) confirm TERAO'S findings, and both writers have postulated that two types of green are responsible for the odd results. Although the inheritance of the green seed coat was independent of the paternal parent yet two types of greens were distinguished in the F_2 progenies of green seed-coat ♀ × yellow seed-coat ♂. One type was more intense than the other and when counts were made it was found that the results were approximately in accord with a ratio of 3 dark green to 1 light green. One type of green was therefore inherited according to Mendelian expectation while the other type was a clear case of maternal inheritance.

ALLELOMORPHIC SERIES FOR THE RESTRICTION OF PIGMENT IN THE SEED COAT

NAGAI and SAITO (1923) postulated that the three restriction factors which they described as *H*, *I*, and *K*, were allelomorphic. This assumption has been well confirmed in the present study, so there seems to be no good reason for not making this clear in the terminology. Four allelomorphs may, therefore, be described according to their inhibiting effect upon the production of pigment in the seed coat. The following figure illustrates the action of the factors which have been found in this allelomorphic series.



As the above figure suggests I^h, under the proper environmental conditions, inhibits all pigment formation in the seed coat; I^i inhibits pigments to the hilum; I^k inhibits the formation of pigment in such a way as to form the "eyebrow" or "patched" pattern; and *i*, the recessive allelomorph, has no effect.

Although it is necessary to define these restriction factors rather definitely, it should be clearly kept in mind that environmental conditions unfavorable for pigment formation are necessary in order for these restriction factors to fully express themselves. Varieties with the genetic constitution *i i* come under Class I or II of the "Seed-coat Color Types." In these two classes there is no restriction of the pigment and environment has practically no effect but in classes III, IV, and V, which I^kI^k, I^iI^i, and I^hI^h represent respectively, environmental conditions are very important in addition to the hereditary factors. Thus, varieties which come in Class V are of constitution I^hI^h, but may develop considerable mottling. From individual seeds it would frequently be impossible to distinguish the three types because of the variation due to environment but when all the seeds from a plant are available for classification there is usually no difficulty. It should be mentioned, however, that these restriction factors are only partially dominant. A cross between I^hI^h and *i i* invariably

produces an F_1 that is mottled more than plants homozygous for I^h and in such cases it is sometimes difficult to distinguish the color types.

Unfortunately the writer was not successful in obtaining a cross between varieties of constitution $i\,i$ and I^kI^k but NAGAI and SAITO (1923) have already described this relationship. All other combinations, however, have been made and the results from these crosses constitute a critical test for the theory that I^h, I^i, I^k, and i are allelomorphic. The best evidence for this relationship rests upon the results of the three combinations of crosses between Mandarins (I^hI^h), Soysotas ($i\,i$) and Manchus (I^iI^i). The parental plants of the Mandarin and Soysota varieties were from strains selected which were known to be homozygous, and the Manchu selection (No. 59), which will be referred to here, was also known to be homozygous.

The distribution of dominant and recessive types in all F_2 progenies closely approached a 3:1 ratio and the crucial proof for the allelomorphic series rests upon this simple but consistent behavior. The following tabulation indicates this relationship.

	Dominant	*Recessive*	*Deviation*
Mandarin (I^hI^h)×Soyosota ($i\,i$)	15	5	0.0±1.3
Manchu (I^iI^i)×Soyosota ($i\,i$)	250	92	6.5±5.4
Mandarin (I^hI^h)×Manchu (I^iI^i)[1]	223	75	2.0±5.0

Any explanation other than that of an allelomorphic series is obviously precluded because this is the only way in which a 3:1 ratio can be secured for each of the combinations. If the Mandarin variety were of constitution *HHii* and if the Manchu were *hhII* (*H* being synonymous with I^h and *I* synonymous with I^i) the crosses with the Soysota variety could be explained because it may have the constitution *hhii*. Such a theory is shattered when the Mandarin is crossed with the Manchu because with two factors involved there would be a dihybrid ratio and 1/16 of the plants in F_2 would be self-colored, but none were observed. The probability of this happening when 412 plants are grown would be $(15/16)^{412}$ or the odds would be over 10^{11}:1, and results from other similar crosses are also against a two factor difference.

The following crosses were also obtained in which the factor I^k is involved. In the cross Black Eyebrow (I^kI^k)×Glabrous No. 5 (I^iI^i), 186 plants produced seed with the pigment restricted to the hilum and 65 produced seed having the eyebrow pattern. Based on a 3:1 ratio the

[1] There were 412 F_2 plants in this cross but only tawny-pubescent plants were classified for reasons which will follow in the text.

deviation is 2.3 ± 4.6. If I^k were not an allelomorph of I^i then the Glabrous No. 5 parent would necessarily have had the two dominant factors I^i and I^k. Such an assumption is not in accord with the breeding facts from crosses between varieties with pigment restricted and self-color because in every case a single factor difference explains the results.

In order to furnish further proof for the allelomorphic series it would have been desirable to have backcrossed an F_1 plant of constitution I^hI^i or I^hI^k to the self-colored $i\,i$ variety. Such crosses should bring out only the two dominant types in the next generation, and the proof for the allelomorphic series would be complete. Due to the difficulty in crossing soybeans, however, this task would be difficult to carry out and the indirect evidence which is already available is practically conclusive that I^h, I^i, I^k, and i occupy identical positions on the chromosomes.

Restriction of pigment to the hilum

It is impossible to assert definitely that a factor identical with NAGAI and SAITO's factor I has been found, but at least it is convenient to use such a designation. This factor I^i, as it is being defined here, is responsible for restricting pigments to the hilum.

The Manchu variety has a yellow seed coat with a black hilum and results have shown that the reason for the seed coat being yellow instead of black is due to the presence of I^i, the dominant restriction factor. Environmental factors produce great variations in the amount of mottling developed; but the hilum remains colored in spite of the fact that environmental conditions may be very unfavorable for the development of mottling.

With no restriction factors other than I^i present the hilum is always black or brown, but the intensity of the color may depend on other factors. In tawny-pubescent varieties the hilum color is prominent but in gray-pubescent varieties the color may be very faint. Varieties differ also in regard to size of hilum and this likewise seems to determine the prominence of the color. Of course, we can not be absolutely sure from the data now available that the factor responsible for restricting pigments to the hilum has been the same one in every case, but neither have we proof for the contrary. It seems logical, therefore, to assume that I^i is responsible for restricting pigments to the hilum.

Three different strains of Manchus were crossed with self-browns but each of these combinations gave very similar results. Table 4 gives the detailed behavior of these crosses but the combined results obtained in F_2 are the following:

Phenotypes	*Black hilum* R_1I^i	*Brown hilum* r_1I^i	*Self-black* R_1i	*Self-brown* r_1i
Observed	584	169	171	71
Calculated	*559.7*	*186.6*	*186.6*	*62.2*
Difference	+24.3	−17.6	−15.6	+8.8

The value of χ^2 is 5.26 assuming independence between I^i, the restriction factor, and R_1 the factor for blaek pigment. This gives P a value of 0.16.

Another cross already mentioned was made between the Black Eyebrow variety (I^kI^k) and a glabrous Japanese variety (I^iI^i) (No. J5) with a brown hilum. The Black Eyebrow variety is characterized by the eyebrow pattern that has been described so we assume that the restriction factor I^k is present. The factor I^i has the same action with the eyebrow pattern as with the self-color, inhibiting the development of pigment outside of the hilum in both cases. The results are as follows:

Phenotypes	*Black hilum* R_1I^i	*Brown hilum* r_1I^i	*Black eyebrow* R_1I^k	*Brown eyebrow* r_1I^k
Observed	136	50	47	18
Calculated	*141.2*	*47.1*	*47.1*	*15.7*
Difference	−5.2	+2.9	−0.1	+2.3

It may be safely assumed that I^i and R_1 were independent because of the small deviations from the theoretical 9:3:3:1 ratio and the value for χ^2 is 0.329 and P = 0.9+.

Restriction of pigment over the entire seed coat

NAGAI and SAITO (1923) have described a factor H which completely inhibits the development of brown and black pigments. They neglect to state that mottling is possible when H is present but there are many reasons to believe that such is the case. The writer's experience has been that the expression of this factor, I^h according to the new usage, may be greatly influenced by environmental conditions. All pigment may be entirely restricted or there may be considerable mottling. The Mandarin, Aksarben, and Ito San varieties have such restriction factors and these three varieties have been crossed rather extensively.

When a self-color (brown or black) is crossed with a variety possessing the restriction factor I^h, the F_1 has a strong tendency to be intermediate. Even in the greenhouse, where conditions extremely unfavorable for the production of mottling were provided, heterozygous plants produced seeds slightly speckled or blotched with pigment. This condition was not apparent, however, when the cross was made with the Manchu variety

which possessed the factor I^i for the restriction of pigment to the hilum. It is to be assumed, therefore, that with both I^i and I^h present the tendency for a restriction of pigment is stronger than if only one I^h is present.

In order to have a convenient method of referring to pigment patterns the designations used in the classification of color types have been adhered to. "Dark hilum," therefore, means that the hilum is colored as in the Manchu variety but the pigment may be black or brown. The factor I^i is responsible for inhibiting the pigment to the hilum in this manner. "Light hilum" means that I^h is present and no pigment is formed except that which may be due to mottling.

No difficulty was experienced in classifying tawny-pubescent plants according to whether their seeds had dark or light hilums, but with gray-pubescent plants the distinction has not always been clear because of the faint development of pigment. In some way the presence of T seems to intensify seed-coat pigments and this helps materially in classifying plants for the presence or absence of I^h.

Four different combinations between self-browns and yellows have been made. The detailed results are given in Table 5 but the combined results are the following:

Phenotypes	*Tawny mottled* TI^h	*Tawny self-brown* Ti	*Gray mottled* tI^h	*Gray, Buff* ti
Observed	137	48	38	22
Calculated	*137.8*	*45.9*	*45.9*	*15.3*
Difference	−0.8	+2.1	−7.9	+6.7

A comparison with calculated results, on the basis of independence, gives deviations that may be accounted for as being due to chance; the value of χ^2 is 4.39 and $P = 0.23$.

Six combinations between black-seeded varieties and varieties possessing the factor I^h were also secured (table 1). The combined F_2 data from these crosses are as follows:

Mottled or colorless	*Self-colored*	*Dev.*	*P. E.*	$\frac{Dev.}{P.E.}$
518	186	10.0	7.75	1.3

These data again show a fairly good agreement between observed figures and the theoretical 3:1 ratio.

A cross, Black Eyebrow $(I^kI^k) \times$Ito San (I^hI^h), was also made which gave an actual ratio of 18 with pigment restricted to 4 with the eyebrow pattern. These data are almost in perfect agreement with theoretical figures, the deviation being 1.5 ± 1.4.

A cross between a Manchu No. 1 (I^iI^i) and an unidentified yellow variety (I^hI^h), designated as No. 12 in the records, gave the following results in F_2.

	LIGHT HILUM		DARK HILUM	
	Black	Brown	Black	Brown
Observed	120	36	51	14
Calculated	*124.4*	*41.4*	*41.4*	*13.8*
Difference	−4.4	−5.4	+9.6	+0.2

Comparing the observed data with the calculated, assuming that I^h is independent of R_1, $\chi^2=3.10$ and $P=0.38$. The behavior of F_3 progenies also corroborates the results obtained in F_2 and these data are given in table 6.

Six combinations between light hilum and gray pubescence ($t\ I^h$) × dark hilum and tawny pubescence ($T\ I^i$) were secured. From a general observation of these F_2's one would naturally assume that I^i is completely linked with T, because no plants with gray pubescence bore seed with the typical dark hilum. If complete linkage is assumed we have the following results compared with the theoretical 2:1:1 ratio.

	Tawny, Light hilum	*Tawny, Dark hilum*	*Gray, Light hilum*	*Gray, Dark hilum*
Observed	709	235	299	
Calculated	*621.5*	*310.7*	*310.7*	
Difference	+87.5	−75.7	−11.7	

With this comparison $\chi^2=31.20$ and $P=0.000001$, so it is unlikely that the deviations could be due to chance. Since T, the factor for tawny pubescence, has a marked effect on both the quantity and quality of pigments, one may assume that some gray-pubescent plants really had dark hilums but could not be easily distinguished. Recalculating with this assumption we get the following comparison with the 9:3:4 ratio.

	Tawny, Light hilum	*Tawny, Dark hilum*	*Gray, (Hilum color not distinguished)*
Observed	709	235	299
Calculated	*699.2*	*233.1*	*310.7*
Difference	+9.8	+1.9	−11.7

In this case $\chi^2=0.59$ and $P=0.9$, showing a very good agreement between observed and expected results.

A very similar cross between the dark and light hilums has been described by WOODWORTH (1921) but he was unable to classify his F_2 plants, because of the irregular pigment which was probably due to environmental conditions.

Restriction of pigment to form an eyebrow pattern

Although the three restriction factors I^h, I^i, I^k seem well established, there are some facts which indicate a larger series of allelomorphs. It has already been mentioned that some strains of Manchus have been selected which mottle much more badly than others, but selection within any one strain has not been successful. In other words these strains must have been homozygous to begin with, and if mutations occur they have not been with sufficient frequency to be selected. In progeny tests with selections from the Black Eyebrow variety, however, heritable variations from the normal eyebrow pattern have been observed, indicating the presence of another factor which restricts the pigment less than I^k, but it is possible that modifying factors were involved.

One selection (No. 84) bred true for the normal eyebrow pattern for four generations and the uniformity was very remarkable. Another selection (No. 82) bred true for a very dark pattern, and in this progeny plants with half of their seeds completely black were not uncommon, but no plant produced 100 percent black seeds. A third progeny (No. 83) was apparently heterozygous for factors affecting the eyebrow pattern because selections were made which bred true for a normal eyebrow pattern; others bred true for a very dark pattern; and still other selections continued to produce some plants with normal patterns and others with dark patterns.

RELATION BETWEEN BLACK AND BROWN PIGMENT

It has been pointed out that self-black is dominant to self-brown in the seed coat and that ratios of 3 blacks to 1 brown are secured in F_2. The first cross made by the writer was of this type and the following results were obtained.

Black	*Brown*	*Dev.*	*P. E.*
72	28	3	2.92

In this cross (Black, No. 3×Brown, No. 30)[1] the F_1 plant bore seed fully as black as the maternal parent, and in the F_2's there was no visible

[1] This progeny also segregated for an unusual type of hilum peculiar to the Soysota variety, the paternal parent. Ordinarily there is a clean separation between the hilum and the funiculus in soybeans, but in this abnormal type the Malpighian cells are arranged in such a way that the funiculus is torn, leaving a rough surface that is very characteristic. This condition is recessive

difference between the intensity of blacks, that is, black was fully dominant to brown.

In 1923 a natural hybrid (Selection No. 60) was accidentally found that segregated according to a ratio of 3 blacks to 1 brown in the progeny that was grown, but all heterozygous plants bore seed slightly speckled with brown. The results were as follows:

	Black	*Dilute black*	*Brown*
Observed	7	14	8
Calculated	*7.25*	*14.50*	*7.25*

It so happened that the observed distribution was exactly in accord with the calculated except for an extra brown. Results in F_3 proved that all of the 14 plants with dilute black were actually heterozygous. The peculiar behavior of the black pigment is noticeable only in the heterozygous condition. In homozygous plants the black is apparently just as intense as that produced by any other variety. It is, therefore, a simple case of incomplete dominance. This kind of partial dominance is well known, and 1:2:1 ratios are perhaps as much to be expected as 3:1 ratios. BLAKESLEE and AVERY (1917) have reported a case of incomplete dominance of mottling in the Adzuki bean (said to be *Phaseolus Mungo*) that is very similar to this lack of dominance in the seed-coat color of the soybean.

The difference between the two kinds of blacks appeared interesting, so crosses were made using the hybrid F_2 plants for parental material. Crosses with types possessing the dominant inhibiting factor I^h were secured. Actually this was equivalent to back-crossing because in some cases heterozygous plants were used as parents. Therefore, combinations with r_1 were secured as well as with R_1.

Two combinations with R_1 are given in table 1, Mandarin and Aksarben varieties being the other parents. Without considering the details of the table at this time it may be of interest to state that out of 17 self-blacks grown in 1925 from these crosses 10 were dilute black and were undoubtedly heterozygous, as was previously found to be the case.

FLOWER AND PUBESCENCE COLORS IN RELATION TO SEED-COAT PIGMENTS

In table 1 six crosses are compared between blacks with a factor present for the production of anthocyanin pigment and yellows with I^h present for the restriction of all pigment. It has already been pointed out that

to the normal hilum and in all crosses it has proved to be independent of all other perceptible characters. Tables 9 and 10 give the evidence for this statement, the factor for normal hilum being designated as N and that of the Soysota variety as n.

black × brown has usually given 3:1 ratios, black being dominant. WOODWORTH'S (1921) example, where complementary factors were involved giving a 9:7 ratio, is the only exception reported.

In the crosses reported in table 1 three strains of blacks (No. 3, No. 29,[1] and No. 60) and four strains of yellows (Mandarins, Ito Sans, Aksarbens, and No. C3) were used. No. C3 is an unidentified Chinese variety, and it resembles the Mandarin variety very much but the flowers are white. It may also be said that this is the only white-flowered variety used in the crosses referred to in table 1. The other six parents had the ordinary purple flowers which are dominant to white.

Black No. 60 was a selection from the natural hybrid that has been described, the peculiar thing about this selection being the incomplete dominance of the black seed-coat color in the heterozygous condition.

Table 1 affords an opportunity for a study of pubescence colors in relation to seed-coat colors. Ito Sans have tawny pubescence while the other three yellow-seeded varieties have gray pubescence. When Ito Sans were crossed with blacks a 9:3:3:1 ratio resulted in F_2 and the results can be explained due to the presence of R_1 in the blacks and I^h in the Ito Sans, but these results are quite different from those obtained when gray-pubescent varieties were used as parental stock. When Mandarins and the Aksarbens were crossed with blacks a 27:9:9:3:9:3:3:1 ratio was found to explain the results. It will be noted that rather high values for χ^2 are obtained for these crosses in table 1 but the numbers are rather small and the fact that all classes are represented may be of some significance. Combining progenies, 133, 139, 172, 175, 171, and 164 $\chi^2 = 14.52$ and $P = 0.04$. Both the black and the brown pigments were influenced in some way by the presence of T. On plants with gray pubescence, of constitution *tt*, there was an incomplete development of pigment. The black and brown correspond with what NAGAI (1921) has described as "imperfect black" and "buff," respectively. It is therefore convenient to continue the use of these terms.

NAGAI does not state that imperfect black was associated with gray pubescence but this has been the case in progenies 133, 172, and 164 (table 1). The logical assumption, therefore, is to consider T identical with NAGAI'S factor C. If these factors are not identical they are at least completely linked, because tawny pubescence has always been associated with a more intense development of both the anthocyanin and phlobaphene-like pigments. These are the exact properties which NAGAI has attributed to his factor C.

[1] Selection from Wisconsin Black variety. Nos. 3 and 60 could not be identified as to variety.

TABLE 1

F_2 progenies of black×yellow seed coats.

	PROGENY NUMBERS	TAWNY PUBESCENCE				GRAY PUBESCENCE				
		Black		Brown		Imperfect black		Buff		
		Mottled	Self-color	Mottled	Self-color	Mottled	Self-color	Mottled	Self-color	
Black (No. 3) ×Ito San	150	13	5	4	0					
Calculated		*13*	*4*	*4*	*1*					$\chi^2 = 1.25$
Difference			+1		−1					$P = 0.74$
Black (No. 29) ×Ito San	127	26	18	11	6					$\chi^2 = 7.24$
Calculated		*34.4*	*11.4*	*11.4*	*3.8*					
Difference		−8.3	+6.6	−0.4	+2.2					$P = 0.07$
	139[3]	72	21	18	10	10	7	7	1	
Black (No. 29) ×Mandarin	133	31	7	6	7	8	3	2	2	
	Total	103	28	24	17	18	10	9	1	$\chi^2 = 14.89$
Calculated		*88.6*	*29.5*	*29.5*	*9.8*	*29.5*	*9.8*	*9.8*	*3.3*	
Difference		+14.4	−1.5	−5.5	+7.2	−11.5	+0.2	−0.8	−2.3	$P = 0.04$
	175	30	11	19	3	6	3	4	0	
Black (No. 60) ×Mandarin	172	26	6	10	2	5	3	7	3	
	Total	56	17	29	5	11	6	11	3	
Calculated		*58.2*	*19.4*	*19.4*	*6.5*	*19.4*	*6.5*	*6.5*	*2.2*	$\chi^2 = 12.56$
Difference		−2.2	−2.4	+9.6	−1.5	−8.4	−0.5	+4.5	−0.8	$P = 0.08$
	171[3]	31	13	14	4	9	3	10	2	
Black (No. 60) ×Aksarben (No. 53)	164	25	11	10	0	8	2	4	2	
	Total	56	24	24	4	17	5	14	4	
Calculated		*62.4*	*20.8*	*20.8*	*6.9*	*20.8*	*6.9*	*6.9*	*2.3*	$\chi^2 = 12.64$
Difference		−6.4	+3.2	+3.2	−2.9	−3.8	−1.9	+7.1	+1.7	$P = 0.08$
Black (No. 3) ×Yellow (No. C3)	142	70	25			15[2]	4[2]	7[1]	4[1]	$\chi^2 = 3.21$
Calculated		*70.3*	*23.4*			*17.6*	*5.9*	*5.9*	*2.0*	
Difference		−0.3	+1.6			−2.6	−1.9	+1.1	+2.0	$P = 0.67$

[1] Plants also had white flowers.

[2] All plants had purple flowers.

[3] Progenies 139 and 171 are reciprocals of 133 and 164, respectively.

Progeny No. 142 [black No. 3 (r_1R_2TW)×yellow No. C3 (r_1r_2tw)] is of special interest because no browns appeared in the tawny-pubescent class, but in the gray-pubescent class there were both imperfect blacks and buffs. This gives a ratio of 15:1 for the presence versus absence of anthocyanin pigment. In the gray-pubescent class the imperfect blacks and buffs were very similar to seed similarly classified in the progenies 133, 172, and 164. F_3 progenies were also grown from F_2 plants of progeny 142. The original hypothesis was confirmed by these results. Although only 10 progenies from plants of phenotypic constitution (T R_2I^h) were grown yet every possible genotypic arrangement was secured. Two segregated the same as the previous F_2 generation, five segregated for I^h but not for T, and three segregated for T but not for I^h. In the later progenies, R_2 was linked completely with T and there were no signs of crossing over.

It is of special interest to note that the imperfect blacks in progeny 142 were also associated with purple flowers and that buffs were associated with white flowers. Therefore, it seems entirely probable that W, the factor for purple flowers (WOODWORTH 1923), may also be responsible for the development of anthocyanin pigment in the seed coat in this particular case. To obtain further evidence F_3 progenies of phenotypic constitution T R_2iw were grown in 1926 and two of these proved to be heterozygous for T. The linkage between T and R_2 was again complete and all gray-pubescent plants produced buff seed-coat color. This evidence corroborates the previous breeding behavior in showing the necessity of the presence of W before anthocyanin pigment can be produced in the seed coat of gray-pubescent plants.

WOODWORTH found that purple flower color was associated with purple hypocotyls but no effect on the seed coat has been reported; yet this condition is not surprising because in other plants the development of anthocyanin pigment has often been found to be associated with flower color. COMBES (1912) working with a species of Spiraea, found that coloration in the bark of twigs was easily brought about by decortication on species with colored flowers but this was not possible with species having white flowers.

As already stated, the crosses Black×Mandarin or Black×Aksarben can readily be explained by the three factors, R_1, T, and I^h. The restriction factor I^h is independent of the other two factors, so there is no need of considering it at this time. The expression of the other two factors is as follows:

R_1T	Black, tawny
R_1t	Imperfect black, gray
r_1T	Brown, tawny
r_1t	Buff, gray

In the cross, Black (No. 3) × Yellow (No. C3), the situation is also simple but there is difficulty in explaining why the behavior is so different from other crosses. It cannot be said that R_1 is responsible for the production of anthocyanin in this case because of the complete linkage. Perhaps we can postulate that another factor R_2, not allelomorphic to R_1, is the factor for anthocyanin formation and that it is completely linked with T. This factor may be identical with Woodworth's factor H because H also produced black pigment and was completely linked with T. The factor W has no apparent effect when T is present but when the constitution was tt it produced an imperfect black. The question then arises—why is it possible that W can have this effect? If it had produced the same effect in Mandarins and Aksarbens the seed coats on these varieties should have been mottled with imperfect black instead of light brown because these are purple-flowered varieties. The genetic constitution of the yellow selection No. C3 must, therefore, differ from the genetic constitution of Mandarins in such a way that when W is present a certain amount of brown pigment is converted into anthocyanin. Perhaps r_2, an allelomorph to R_2, makes this possible; and if this is the case a cross between the Mandarin (with W) and selection No. C3 (with r_2) should give imperfect blacks in F_1; i.e., the complementary factors W and r_2 are necessary to explain the results.

On the basis of the above assumption the black selection No. 3 (r_1R_2TW) must have a factor R_2 which is different from the factor R_1 in No. 29 and No. 60, and selection No. C3 (r_1r_2tw) must also have an additional factor r_2 that is not present in Mandarins and Aksarbens. Then the results in progeny 142 can be explained as follows:

r_1R_2TW, r_1R_2Tw ..... Black, tawny
r_1r_2tW ..... Imperfect black, gray
r_1r_2tw ..... Buff, gray

Since r_2 was completely linked with t, the single factor T may be assumed to be responsible for the production of black seed coats as well as tawny pubescence, but results from other crosses show that T does not always have this effect. When selection No. 3 (r_1R_2T) was crossed with the Ito San ($r_1r_2'T$) there were plants with brown seed coats and tawny pubescence in F_2 (table 1). Furthermore, r_2' of the Ito San variety must be considered different from r_2 of selection No. C3 because r_2 was a complement of W for the production of anthocyanin in the imperfect black seed-coats of progeny 142 (table 1), but r_2' does not have this effect. From

these results it seems possible that an allelomorphic series may be situated at the *t* locus. If this is true the following combinations have now been identified:

R_2T.....Tawny pubescence, black seed-coat (selection No. 3)
r_2t.....Gray pubescence, seed-coat imperfect black with *W* but buff with *w* (selection No. C3).
r'_2T.....Tawny pubescence, seed-coat brown with either *W* or *w* (Ito San variety)
r'_2T.....Gray pubescence, seed-coat brown with *W* or *w* (Mandarin and Aksarben varieties).

The identity of the factor R_2 for the production of anthocyanin pigment in the seed coat is well established, but it has been found in only one selection (No. 3). In selection Nos. 29 and 60 the factor (R_1) responsible for the production of anthocyanin pigment in the seed coat is clearly distinct from R_2, because, unlike the latter, it is not linked with *T*. There is also good reason for using the designation R_1 in selection No. C4 (table 3) but a number of other combinations were made between varieties both of which had tawny pubescence. In these crosses the factor for the production of anthocyanin may have been either R_1 or R_2 but for convenience R_1 has been used in case of doubt.

DUPLICATE FACTORS FOR TAWNY PUBESCENCE

The assumptions that have just been made depend upon *T* being the same factor for tawny pubescence in each case. It might have been assumed that there is only one factor *R* for black seed-coat color and two factors for tawny pubescence. A convenient designation would be T_1 and T_2. The factor T_1 could be independent of *R*, but *R* and T_2 would be completely linked. Such an assumption would explain the results as well as the method which has been chosen.

Further work is necessary to decide these relationships but the results from one F_2 population give a real indication for the presence of two factors for tawny pubescence. This was progeny No. 71 (No. Sp 3-9 × No. 29) which has been discussed under Part I of this series, as evidence for maternal inheritance of cotyledon color. Both paternal parents were homozygous for tawny pubescence but there was segregation in F_2 giving 71 tawny to 15 gray pubescent plants. Assuming duplicate factors the deviation is 10.6 ± 4.8. It is very evident that this is a rather wide deviation from a 15:1 ratio but since the odds are only about 6:1 it does not seem entirely improbable. F_3 progenies from this cross are to be grown and results reported at a future date.

LINKAGE *versus* INDEPENDENCE OF SEVEN PAIRS OF FACTORS

Eleven crosses between Mandarins and an unidentified Chinese variety, given the arbitrary number C4, proved successful. These varieties differ in seven pairs of characters. These contrasted characters and the factors which correspond to them are as follows:

Mandarin	*Green No. C4*
Purple flower color, W	White flower color, w
Yellow pods, l	Black pods, L
Gray pubescence, t	Tawny pubescence, T
Yellow seed-coat, g	Green seed coat, G
Light hilum, I^h	Dark hilum, I^i
Brown mottling pigment, r_1	Black mottling pigment, R_1
Yellow cotyledon color, D_1D_2	Green cotyledon color, d_1d_2

The factor pairs Gg, I^hI^i, and R_1r_1 have the same meaning here as in NAGAI and SAITO'S (1923) descriptions; and Ww, Ll, Tt, and D_1d_1, have been described by WOODWORTH (1921 and 1923). The factor D_2 is a duplicate of D_1 for the production of yellow cotyledons, and is identical with WOODWORTH'S factor I, the only reason for the change being the use of I in designating a restriction factor.

The action of R_1 in this cross was the same as has been previously described. The dark hilum was easily distinguished from light hilum on tawny-pubescent plants, but the intensity of pigment was reduced on gray-pubescent plants, making it difficult to detect the presence of the restriction factor. In fact, all the gray-pubescent plants bore seed so much lighter in color than the tawny-pubescent plants that all were classified as having light hilums. Therefore only 3/16 of the total number of plants should come in the dark hilum class, and a 13:3 ratio should be expected.

The seed-coat color due to plastid pigment was perhaps the most variable character. The F_1 seed coats produced a green color but it was somewhat intermediate. F_2 results were in very close agreement with a 3:1 ratio but there were all gradations in intensity of green. The pod color was no doubt responsible for much of this variation because light-colored pods were always associated with a "faded-out green." Perhaps this can be explained as a result of the plants remaining in the field in the presence of bright sunlight for a considerable length of time after ripening. Black-podded plants always produce a normal green or yellow seed coat, and it seems possible that this can be accounted for by attributing to the black color the absorption of the sunlight which changes normal green seed

coats to a yellowish green. In the light-colored pods the same effect was also produced on green cotyledons as described in Part I of this series.

There was a close approach to a 15:1 ratio for cotyledon color in all F_2 seed. The total numbers were 1686 with yellow cotyledons to 118 with green cotyledons, so the deviation is only 5.25 ± 6.9 from the expected numbers. Usually the seeds were easy to classify because the difference was clear-cut. Both of the parental varieties matured well in Wisconsin and no cotyledons were green due to immaturity. The F_1 plants also produced black pods, so there was no tendency for the cotyledon color to fade as it did on certain F_2 plants with yellow pods.

The parental variety No. C4 has very dark brown or black pods. As has been stated the pigment responsible for this coloration has no relation to the anthocyanin or the brown phlobaphene-like pigment; yet very frequently, on black-podded plants, much of this pigment finds its way into the seed coat and produces a smudgy-brown coloration. This condition has often masked other pigments to such an extent that the classification of seed-coat colors has been very difficult. Fortunately the F_2's were grown on soil which brought out considerable mottling, but it was frequently necessary to treat the seed coats with acid in order to be sure of the classification. This test proved a great aid because a small amount of anthocyanin can be detected by the red color produced in acid solution.

TABLE 2

F_2 progenies of Mandarin×Green No. C4.

	OBSERVED NUMBERS	DEVIATION	$\frac{\text{DEV.}}{\text{P. E.}}$	ACTUAL RATIO
Green *versus* Yellow seed-coat	252:85	0.75	0.14	2.96:1
Tawny *versus* Gray pubescence	262:75	9.25	1.73	3.49:1
Anthocyanin *versus* Brown pigment	251:86	1.75	0.33	2.92:1
Black *versus* Yellow pods	238:99	14.75	2.75	2.40:1
Purple *versus* White flowers	251:86	1.75	0.33	2.92:1
Light *versus* Dark hilum (calculated on basis of 13:3)	265:72	8.8	1.82	11.04:3
Yellow *versus* Green cotyledons	321:16			
(corrected figures)	315.9:21.1	5.1	1.70	20.06:1

Table 2 gives the observed data for each of the contrasted characters of 337 F_2 plants. There is good agreement with expectation among all seven of these contrasted characters when considered separately except black versus yellow pods. This discrepancy perhaps can be accounted for by the fact that the classification was made from a few pods after thresh-

ing. Since the character is somewhat variable, further tests would be necessary to prove that there is any other reason for the results not being in accord with a 3:1 ratio.

In order to determine whether or not there was any linkage, each contrasting pair of characters has been considered separately with every other pair. Table 3 gives these data in a condensed form. Twenty-one combinations are possible and the actual ratios as well as the theoretical are given in the table. The values of χ^2 and P are also included.

TABLE 3

F_2 progenies of Green No. C4×Mandarin—total of 337 plants.

			THEORETICAL RATIO WITH INDEPENDENCE	ACTUAL RATIO	χ^2	P
D_1D_2	*versus* d_1d_2[1]	and *W versus w*	45:15: 3:1	44.3:15.7: 3.5:0.5	2.07	0.56
"		and *T versus t*	45:15: 3:1	46.2:13.8: 3.8:0.2	4.68	0.20
"		and R_1 *versus* r_1	45:15: 3:1	44.7:15.3: 3.0:1.0	0.05	0.9+
"		and *L versus l*	45:15: 3:1	41.9:18.1: 3.5:0.5	6.45	0.09
"		and I^h *versus* I^i	195:45:13:3[2]	192.2:51.7: 9.1:3.0	1.83	0.61
"		and *G versus g*	45:15: 3:1	44.1:15.9: 4.0:0.0	7.44	0.06
G	*versus g*	and *W versus w*	9: 3: 3:1	8.7: 3.2: 3.2:0.9	1.21	0.75
"		and *T versus t*	9: 3: 3:1	9.1: 2.9: 3.3:0.7	2.69	0.45
"		and R_1 *versus* r_1	9: 3: 3:1	9.3: 2.7: 2.6:1.4	4.54	0.21
"		and *L versus l*	9: 3: 3:1	8.7: 3.3: 2.6:1.4	5.58	0.14
"		and I^h *versus* I^i	39:13: 9:3[2]	37.6:12.7:10.3:3.4	1.52	0.68
I^h	*versus* I^i	and *W versus w*	39:13: 9:3	36.8:13.4:10.8:2.8	2.71	0.44
"		and *T versus t*	9: 3: 4:	9.0: 3.4: 3.6:	2.25	0.33
"		and R_1 *versus* r_1	39:13: 9:3[2]	36.6:13.6:11.0:2.7	3.50	0.33
"		and *L versus l*	39:13: 9:3	35.5:14.8: 9.7:4.0	4.95	0.18
L	*versus l*	and *W versus w*	9: 3: 3:1	8.4: 2.9: 3.5:1.2	3.48	0.33
"		and *T versus t*	9: 3: 3:1	8.7: 3.8: 2.6:0.9	5.30	0.15
"		and R_1 *versus* r_1	9: 3: 3:1	8.3: 3.6: 3.0:1.1	3.89	0.28
R_1	*versus* r_1	and *W versus w*	9: 3: 3:1	8.6: 3.3: 3.3:0.8	2.17	0.54
"		and *T versus t*	9: 3: 3:1	9.4: 3.0: 2.5:1.0	2.07	0.56
T	*versus t*	and *W versus w*	9: 3: 3:1	9.3: 3.2: 2.6:0.9	1.41	0.71

[1] The observed ratio was corrected for a 15:1 ratio for cotyledon color in making comparisons with the calculated figures. This is legitimate and advisable because no effort was made to grow these seeds to maturity in exactly that proportion.

[2] These odd ratios are due to the fact that all gray-pubescent plants were classified as having light hilums. Really ¼ of these plants had dark hilums but they could not be readily identified.

It will be noted that five combinations give values of P less than 0.2 but four of these are combinations with black versus yellow pods where

the original ratio was 2.4:1; rather poor fits[1] with other pairs of characters, therefore, would naturally result. One combination alone (plastid color in the seed coat versus cotyledon color) appears to be a case of linkage. In this case the value of P is 0.06 which means that the odds are only about 16:1 against such a fit being due to chance but an examination of the actual results indicates that the odds may be greater. This linkage relation has been discussed in Part I of this series.

LINKAGES

R_2, factor for black pigment in seed coat (Perhaps identical with WOODWORTH's *H*) *T*, factor for tawny pubescence.	Completely linked.
T, factor for tawny pubescence. *E*, factor for early maturity.	Since the expression of *E* is somewhat quantitative the linkage could not be accurately measured, but there is proof for only a small percentage of crossing over.
P_1, factor for glabrousness. R_1, factor for black pigment in seed coat.	Linkage appears to be almost complete with limited data.
P_1, factor for glabrousness. *M*, factor for black mottling.	About 18 percent crossing over. (NAGAI and SAITO 1923)
G, factor for green seed coat, designated as *V* by WOODWORTH D_1, factor for yellow cotyledon color	About 13 percent crossing over. (WOODWORTH 1921. Also the present study.)

Three linkage groups are necessary to explain the above linkages, but the factors cannot be arranged according to their respective positions. These groups are as follows:

$$R_2-T-E$$
$$P_1-R_1-M$$
$$G-D_1$$

LIST OF GENETIC FACTORS

The genetic factors used to explain the characters which have been studied in soybeans are as follows:

T, factor for tawny pubescence. Also increases the intensity of color in the seed coat; *t*, gray pubescence and a lighter seed-coat color. (WOODWORTH 1921) (Limited evidence indicates that there may be another factor which produces the same results as *T* but is independently inherited.)

W, factor for purple flower and hypocotyl color and also a complement of r_2 for the production of black pigment in the seed coat; *w*, white flowers and green hypocotyls. (WOODWORTH 1923.)

[1] A preferable method of calculating x^2 as a measure of linkage follows this article.

R_1, factor for black pigment in the seed-coat but without T the color is imperfect black; r_1, brown pigment but without T the color is light brown or buff. (NAGAI 1921.)

R_2, also a factor for black pigment in seed coat, but entirely linked with T (possibly this factor is identical with WOODWORTH'S factor H); r_2, brown pigment (but is a complement of W. With r_2r_2 and W present, the pigment is imperfect black in appearance); r_2', brown pigment but not complementary to W.

B, found to be a complementary factor to Woodworth's H for the production of black pigment in the seed-coat. (WOODWORTH 1921.)

O, factor for dull brown seed-coat color; o, reddish brown. (NAGAI 1921.)

I, factor which inhibits pigment formation in the seed-coat—an allelomorphic series. i, has no effect.

I^k, inhibits pigment to form an eyebrown pattern (identical with NAGAI and SAITO'S, 1923, factor K).

I^i, inhibits pigment to the hilum—dominant to I^k and i. (Possibly the same as the factor I which NAGAI, 1921, describes as nearly completely inhibiting the formation of chromogenic substance in the seed-coat.)

I^h, inhibits pigments entirely except that which may be due to mottling—dominant to I^i, I^k, and i. (Identical with NAGAI and SAITO'S factor H.)

M, factor for the production of black mottling upon a self-brown seed-coat; m, no effect. (NAGAI and SAITO 1923.)

G, factor for green seed-coat; g, yellow seed coat due to the green plastid color fading out at maturity. (NAGAI, 1921, also WOODWORTH, 1921, but V was used for the symbol instead of G.)

D_1, factor for yellow cotyledon color; d_1, no effect. (WOODWORTH 1921.)

D_2, duplicate of D_1, for causing the green cotyledon color to fade out at maturity (identical with WOODWORTH'S factor I); d_2, no effect.

N, factor for normal hilum; n, abnormal hilum as in the Soysota variety.

P_1, factor for glabrousness; p_1, pubescence. (NAGAI and SAITO 1923.)

P_2, factor for pubescence, p_2, glabrousness (relationship to P_1 and p_1 not known as yet except that the order of dominance is reversed)—STEWART and WENTZ (1926).

L, factor for black pods but also causes a black coloration of the seed-coat in certain instances; l, brown pods. (WOODWORTH 1923.)

S, factor for tall plant; s, short plant. (WOODWORTH, 1923, also reported by the writer in table 8.)

E, factor for early maturity; e, late maturity.

In the above list of factors reference is made to all original descriptions of previous investigators. Those factors which do not have such a reference are described here for the first time. Of course, there is no definite proof that the factors which are considered synonymous are really identical, but it seems advisable to consider them as such until there is proof for the contrary.

SUMMARY

In all the crosses that have been made black seed coat has been dominant to brown but in one cross the dominance was incomplete. Results also indicate that different factors may produce black pigment; and a genetic interpretation has been given to account for the presence of R_1

and R_2, each of which is capable of producing anthocyanin pigment in the seed coat.

In one particular cross, the factor W, for purple flowers, was found to be a complement of r_2 for the production of black pigment in the seed coat; but in all other cases purple-flower color was independent of every other character.

Three restriction factors have been described. These are dominant factors but their expression is very sensitive to the conditions under which the plant is grown. An environment unfavorable for the production of mottling is necessary for pigment to be completely restricted; otherwise there may be considerable mottling in spite of the presence of restriction factors.

The factor T, for tawny pubescence, has proved to be very important in increasing the intensity of pigment. This is perhaps due to an actual increase in the amount of pigment.

In black-podded varieties a certain amount of the brownish-black oxidation pigment of the pod was sometimes evident in the seed coat.

Five linkages are postulated. The crossover percentage between G, the factor for green seed coat, and D, a factor for yellow cotyledons, was approximately 13 percent, the same as WOODWORTH (1921) has reported. The factor T, for tawny pubescence was completely linked to R_2. All other linkages are quite tentative until more data are secured.

A complete list of all the genetic factors studied in soybeans has been given as well as those directly concerned with seed-coat colors.

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APPENDIX

TABLE 4

F_2 progenies of brown (r_1i)×yellow with black hilum (R_1 I^h).

	PROGENY NUMBERS	DARK HILUM		SELF-COLORED SEED-COAT		
		Black R_1I^i	Brown r_1I^i	Black R_1i	Brown r_1i	
Soysota (Brown No. 30) ×Manchu (No. 59)	213	58	14	18	9	$\chi^2=2.53$
	224	53	13	13	6	$P=0.47$
	217	84	28	33	13	
	Total	195	55	64	28	
Calculated		*192.4*	*64.1*	*64.1*	*21.4*	
Difference		+2.6	−9.1	−0.1	+6.6	
Brown (No. 12)×Manchu (No. 1)	186	71	11	18	5	$\chi^2=4.09$
	190	128	41	38	11	$P=0.25$
	Total	199	52	56	16	
Calculated		*181.7*	*60.6*	*60.6*	*20.2*	
Difference		+17.3	−8.6	−4.6	−4.2	
Brown (No. 12)×Manchu (No 16) *Calculated*	8	190	62	51	27	$\chi^2=4.95$
		185.6	*61.9*	*61.9*	*20.6*	$P=0.18$
Difference		+4.4	+0.1	−10.9	+6.4	
Total of F_2 progenies		584	169	171	71	$\chi^2=5.26$
Calculated		*559.7*	*186.6*	*186.6*	*62.2*	$P=0.16$
Difference		+24.3	−17.6	−15.6	+8.8	

TABLE 5

F_2 progenies of yellow (tI^h)×brown seed coat (Ti).

	PROGENY NUMBERS	TAWNY PUBESCENCE		GRAY PUBESCENCE		
		Mottled with brown	Self-brown	Mottled with brown	Self-brown	
Mandarin×Brown (No. 12)	197	40	10	10	6	$\chi^2=2.04$
Calculated		*37.1*	*12.4*	*12.4*	*4.1*	$P=0.57$
Difference		+2.9	−2.4	−2.4	+1.9	
Mandarin×Soysota (Brown No. 30)	231	10	2	5	3	$\chi^2=3.61$
Calculated		*11.3*	*3.7*	*3.7*	*1.3*	$P=0.31$
Difference		−1.3	+1.7	+1.3	+1.7	
Yellow (No. C2)×Soysota (Brown No. 30)	208	68	26	18	9	$\chi^2=1.71$
Calculated		*68*	*22.7*	*22.7*	*7.6*	$P=0.65$
Difference			+3.3	−4.7	+1.4	
Aksarben×Brown (No. 60)	164a	19	10	5	4	$\chi^2=3.14$
Calculated		*21.4*	*7.1*	*7.1*	*2.4*	$P=0.37$
Difference		−2.4	+2.9	−2.1	+1.6	
Total observed		137	48	38	22	$\chi^2=4.39$
Calculated		*137.8*	*45.9*	*45.9*	*15.3*	$P=0.23$
Difference		−0.8	+2.1	−7.9	+6.7	

TABLE 6

Dark black hilum (R_1I^i)×light brown hilum (r_1I^h).

	NUMBER OF PROGENIES	LIGHT HILUM		DARK HILUM		
		Black pigment	Brown pigment	Black pigment	Brown pigment	
F_2 Progeny No. 1 (Manchu No. 1×Yellow No. 12)		120 *124.4* −4.4	36 *41.4* −5.4	51 *41.4* +9.6	14 *13.8* +0.2	$\chi^2=3.10$ $P=0.38$
F_3 Progenies from seeds with light black hilum	1	45	12	16	2	
Deviation from calculated		+2.8	−2.1	+1.9	−2.7	
	1	36		14		
Deviation from calculated		−1.5		+1.5		
	2	49	18			
Deviation from calculated		−1.3	+1.3			
	1	17		7		
Deviation from calculated		−1		+1		
	2	57				
F_3 Progenies from seeds with black dark hilum	1			11	2	
Deviation from calculated				+1.2	−1.3	
	2			76		
F_3 Progenies from seeds with brown light hilum	3		71		26	
Deviation from calculated			+1.8		−1.7	
	2		38			
F_3 Progenies from seeds with dark brown hilum	2				44	

TABLE 7

Tawny-pubescent, Dark black hilum (I^iR_1)×Gray-pubescent, Light brown hilum (tI^hr_1).

	PROGENY NUMBERS	TAWNY PUBESCENCE				GRAY PUBESCENCE		
		Light Hilum		Dark Hilum		Light-dark Hilum		
		Black Pigment	Brown Pigment	Black Pigment	Brown Pigment	Black Pigment	Brown Pigment	
Green (No. C4)×Aksarben	31	20	6	5	3	5	6	
Deviation from calculated		+1.0	−0.3	−1.3	+0.9	−3.4	+3.2	$\chi^2=5.75$ $P=0.33$
Yellow (No. C2)×Manchu (No. 1)	269	10	4	6	4	11	2	
Deviation from calculated		−5.6	−1.2	+0.8	+2.3	+4.1	−0.3	$\chi^2=8.00$ $P=0.16$
Mandarin×Green (No. C1)	342	7	6	9	2	7	2	
Deviation from calculated		−6.9	+1.4	+4.4	+0.5	+0.9	−0.1	$\chi^2=8.33$ $P=0.14$
Mandarin×Manchu (No. 1)	300	161	62	40	14	51	20	
	292	14	6	4	1	5	1	
	Total	175	68	44	15	56	21	
Deviation from calculated		+15.1	+14.7	−9.3	−2.8	−15.1	−2.7	$\chi^2=11.06$ $P=0.05$
Manchu (No. 59)×Mandarin	282	134	47	48	17	71	23	
	272	28	14	7	3	9	11	
	Total	162	61	55	20	80	34	
Deviation from calculated		−11.8	+3.1	−2.9	+0.7	+2.7	+8.2	$\chi^2=3.84$ $P=0.57$
Green (No. C4)×Mandarin	13	140	50	58	14	53	22	
Deviation from calculated		−2.2	+2.6	+10.6	−1.8	−10.2	+0.9	$\chi^2=4.44$ $P=0.49$
Calculated on basis of 27:9:9:3:12:4 ratio	Total	514	195	177	58	212	87	
		524.5	*174.8*	*174.8*	*58.3*	*233.1*	*77.7*	
Difference		−10.5	+20.2	+2.2	−0.3	−21.1	+9.3	$\chi^2=5.60$ $P=0.35$

TABLE 8

Showing independence between S, the dominant factor for height of plant and R_1 and G—Progeny No. 319—No. 57 (Sr_1g)×No. C_1 (sR_1G).

	SGR_1	SGr_1	SgR_1	Sgr_1	sGR_1	sGr_1	sgR_1	sgr_1	
Observed	85	29	24	9	33	8	11	3	χ^2=1.95
Calculated	*85.2*	*28.4*	*28.4*	*9.5*	*28.4*	*9.5*	*9.5*	*3.2*	P=0.96
Deviation	−.2	+.6	−4.4	−.5	+4.6	−1.5	+1.5	−.2	

TABLE 9

Manchu (R_1I^iN)×Soysota (r_1in).

	NR_1I^i	NR_1i	Nr_1I^i	Nr_1i	nR_1I^i	nR_1i	nr_1I^i	nr_1i	
Observed	41	11	8	5	17	7	6	4	χ^2=9.70
Calculated	*41.8*	*13.9*	*13.9*	*4.6*	*13.9*	*4.6*	*4.6*	*1.5*	P=0.22
Difference	−0.8	−2.1	−5.9	−0.4	+3.1	+2.4	+1.4	+2.5	

TABLE 10

Black No. 3 (R_2N)×Soysota (r'_2n).

	R_2N	R_2n	r_2N	r_2n	
Observed	49	23	21	7	
Calculated	*56.25*	*18.75*	*18.75*	*6.25*	χ^2=2.26
Difference	−7.25	+4.25	+2.25	+0.75	P=0.50

CALCULATING LINKAGE INTENSITIES BY PRODUCT MOMENT CORRELATION[1]

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INTRODUCTION

The need for a reliable method of calculating linkage intensities from F_2 populations is apparent from the literature and is appreciated after a minimum of experience. The present contribution is an attempt to make use of well known statistical work by illustrating the relationship between product moment correlation and crossing over. For "theoretical Mendelism" there is no doubt that the Pearsonian product moment coefficient of correlation may be used with entire safety. The "Pearsonian school" (PEARSON and HERON 1913) grants this usage but objects when it is applied to "practical Mendelism."

The whole crux of the matter, therefore, lies in the interpretation of "practical Mendelism." Are phenotypic classes so confluent that gametic values can never be considered as discrete units? One must candidly admit that it all depends upon the particular unit character under consideration, the environment under which it is produced (MORGAN 1919,

[1] Papers from the Biological Laboratory, MAINE AGRICULTURAL EXPERIMENT STATION, No. 180.

page 29) and the skill of the investigator (EMERSON 1921, page 16). The methods of calculating linkage intensities here presented presuppose clear-cut Mendelian segregation, and it should be perfectly obvious that as the classifications become increasingly arbitrary that estimations of linkage intensities become more and more inaccurate.

COLLINS (1924) has described three methods of measuring linkage (EMERSON'S, HALDANE'S and YULE'S) all of which give conflicting results when the observed ratios do not agree exactly with the theoretical. EMERSON'S (1916) method is simple and a table has been worked out by ALBERTS (1926) but the calculations are not based on random deviations from the 3:1 ratio. HALDANE'S (1919) method is valuable but the end results are not always satisfactory. COLLINS maintains that YULE'S coefficient of association Q "most nearly meets the requirements of a general method" but "no one (of the three methods) is found to be most accurate for all classes of departures." More recently FISHER (1925) has outlined a method based upon the best possible fit in comparison with theoretical ratios. Fundamentally the procedure is the same as HALDANE'S but FISHER'S formula is much more convenient for application. This method is especially valuable but is subject to the same criticism which COLLINS has given for HALDANE'S method unless it is accompanied by additional information.

It is the writer's intention that the method which is being introduced should, for certain critical cases, be accompanied by the use of FISHER'S and YULE'S formulae, but for a general method the product moment coefficient of correlation seems the most logical. Not only are the results more consistent and conservative but one gains the additional advantage of convenient algebraic manipulation.

For a 2×2 classification the product moment coefficient of correlation (made possible by assuming clear-cut Mendelian characters) is identical with the square root of the mean square contingency. Thus the simple relationship $\chi^2=nr^2$, since the mean square contingency $\phi^2=\frac{\chi^2}{n}$. This makes it possible to estimate the crossing over percentage from r and also the odds for goodness of fit by χ^2 in one operation. The simple relationship minimizes the labor of calculation and gives a critical test. Furthermore, the method is not confined to correlation between characters determined by single factors but has been readily extended to duplicate and complimentary factors and various other complex relationships and also to simple cases of backcrossing.

It is hoped that the tables[1] which have been prepared from theoretical ratios will serve both as a convenience and a caution. Furthermore the basis for assuming a product moment coefficient of correlation must be constantly kept in mind. When practical observations depart from the theoretical extreme care must be used and a wide field (see KELLEY 1924, page 259) is open for the development of statistical criteria.

THE PRODUCT MOMENT COEFFICIENT OF CORRELATION

A purely theoretical F_2 distribution where $AB=a''$, $Ab=b''$, $aB=c''$, and $ab=d''$ may be arranged in the fourfold table:

	A	a	
B	a''	b''	$a''+b''$
b	c''	d''	$c''+d''$
	$a''+c''$	$b''+d''$	n

Without any knowledge as to the definite value of the qualitative categories A, a, B and b, and without any regard as to the observed ratios or the position of the means, the Pearsonian product moment coefficient of correlation turns out:

$$r^2=\frac{(a''d''-b''c'')^2}{(a''+b'')(c''+d'')(a''+c'')(b''+d'')} \tag{1}$$

by simple substitution in the general equation

$$r=\frac{\Sigma XY-nM_xM_y}{\sqrt{(\Sigma X^2-nM_x^2)(\Sigma Y^2-nM_y^2)}}$$

Since the assumptions are purely theoretical there can be no objection to considering A, a, B, and b as definite abstract values, that is they do not spread over an interval. Hence there is perfect justification for deriving this coefficient of correlation. For critical references see KELLEY 1924, page 259; PEARSON and HERON 1913, page 167; BOAS 1909; YULE 1912; and SNOW 1912.

Relation to χ^2 test for goodness of fit

When $r=0$ it may be assumed that $a''=a'''$, $b''=b'''$, $c''=c'''$ and $d''=d'''$. Then applying the test for goodness of fit

[1] These tables were calculated by Mrs. IVA MERCHANT BURGESS and Miss MILDRED COVELL of the Biological Laboratory of the MAINE AGRICULTURAL EXPERIMENT STATION.

$$\chi^2 = \frac{(a''-a''')^2}{a'''} + \frac{(b''-b''')^2}{b'''} + \frac{(c''-c''')^2}{c'''} + \frac{(d''-d''')^2}{d'''}$$
$$= \frac{(a''d''-b''c'')^2\,(a''+b''+c''+d'')}{(a''+b'')(c''+d'')(a''+c'')(b''+d'')} \qquad (2)$$
$$= nr^2$$

This is but a simple illustration of the well recognized value of χ^2 when the fourfold table is made up according to the specifications of theoretical Mendelism. (See previous references to KELLEY, and PEARSON and HERON).

If, however, the observed values of AB, Ab, aB and ab are not in approximate agreement with the theoretical, that is, when A, a, B, and b fluctuate to such an extent that the whole distribution must be thought of as continuous; then the statistical analysis is very different. The well known tetrachoric r_t has been devised for these cases and the value $\frac{\chi^2}{n}$ is equal to the uncorrected mean square contingency ϕ^2, but with continuous variation ϕ cannot be considered synonymous with the product moment coefficient of correlation.

Determining odds against random distribution

For determining the goodness of fit COLLINS (1924) has already suggested that observed data should be compared with a fourfold distribution based on the observed rather than the theoretical ratios of dominant to recessive phenotypes. This is true if χ^2 is to be a measure of correlation.

To clarify terminology the following definitions may be considered:

		PHENOTYPIC DISTRIBUTION			
		AB	Ab	aB	ab
Observed ratios maintained	Any specific value of r	a	b	c	d
	Correlation reduced to zero	a'	b'	c'	d'
Ratios strictly theoretical (3:1 or 9:7; etc.)	Any specific value of r	a''	b''	c''	d''
	Correlation reduced to zero	a'''	b'''	c'''	d'''

For observed data the fourfold table is:

a	b	$a+b$
c	d	$c+d$
$a+c$	$b+d$	n

and the ratios are $\frac{a+b}{n} : \frac{c+d}{n}$ and $\frac{a+c}{n} : \frac{b+d}{n}$

In the simplest case both of these ratios are approximately 3:1 but much recent evidence (See JONES 1924) clearly shows that various ratios are possible depending upon differential viability of gametes and selective pollen tube growth.

Certainly a comparison with the observed ratios is the more logical method for determining correlation and estimating linkage values. Furthermore such a comparison can be readily made. In fact this is automatically taken care of either in calculating r, the coefficient of correlation or YULE'S Q, the coefficient of association. To determine the uncorrelated distribution which does not change the ratios one must have the following equalities: $a+b=a'+b'$, $c+d=c'+d'$, $a+c=a'+c'$ and $b+d=b'+d'$. Hence,

$$a' = \frac{(a+b)(a+c)}{n}$$

$$b' = \frac{(a+b)(b+d)}{n}$$

$$c' = \frac{(c+d)(a+c)}{n}$$

$$d' = \frac{(c+d)(b+d)}{n}$$

In practise, however, it is necessary to calculate a' only, since the other values can be obtained directly by subtraction in the equalities just given. (See FISHER 1925, page 85).

If it is possible to consider the observed frequencies as discrete units, the product moment coefficient of correlation may be calculated from formula [1] and $nr^2 = \chi^2$. Now the customary practise has been to consult ELDERTON'S tables (PEARSON 1914) to ascertain the probability of observed values of χ^2. No criticism is intended for this procedure but one must remember that the probability secured in this way represents the

odds against a fit as bad as the observed being due to fluctuations in sampling. Obviously a perfect fit is as highly improbable as an extremely bad fit. To ascertain the odds against χ^2, as a measure of correlation, being due to chance is a very different problem. (See KELLEY 1924, page 264).

If it is desired to estimate the odds against random distribution the standard deviation of r deserves serious consideration. This standard deviation is (See YULE 1912, and PEARSON and HERON 1913, page 169)

$$\sigma_r = \frac{1}{\sqrt{n}}\left\{1-r^2+\left(r+\frac{r^3}{2}\right)\left(\sqrt{\frac{q_1}{p_1}}-\sqrt{\frac{p_1}{q_1}}\right)\left(\sqrt{\frac{q_2}{p_2}}-\sqrt{\frac{p_2}{q_2}}\right) - \frac{3r^2}{4}\left(\frac{q_1}{p_1}+\frac{p_1}{q_1}-2\right)\left(\frac{q_2}{p_2}+\frac{p_2}{q_2}-2\right)\right\}^{\frac{1}{2}} \qquad (3)$$

where p_1, q_1, p_2 and q_2 are the observed ratios.

For backcrossing (with theoretical 1:1 and 1:1 ratios) this formula reduces to

$$\sigma_r = \frac{\sqrt{1-r^2}}{\sqrt{n}}$$

and in an ordinary F_2 (with 3:1 and 3:1 ratios)

$$\sigma_r = \frac{\sqrt{1+4/3r-7/3r^2+2/3r^3}}{\sqrt{n}}$$

It is obvious that this standard deviation reduces to $\frac{1}{\sqrt{n}}$ for all possible ratios when $r=0$. For genetic interpretations this is another point of considerable interest because ${}_o\sigma_r$, the standard deviation of r for a random distribution, is the value which is generally desired. It is of further interest that $\frac{r}{{}_o\sigma_r} = \chi = \frac{Q}{{}_o\sigma_q}$ (See PEARSON 1912). This value χ is actually the square root of χ^2 so the convenience is at once apparent. The only unfortunate thing is that the frequency of r in terms of ${}_o\sigma_r$ (the standard deviation with random distribution) probably cannot be considered normal or Gaussian. PEARSON (1912) has given the probability integral for the desired frequency distribution but this awaits further study and development. It is hoped that eventually tables for this probability integral will be available. Then the odds against random distribution can be looked up directly rather than using the probability for goodness

of fit from ELDERTON'S tables. The odds for goodness of fit from ELDERTON'S tables (see COLLINS 1924, for table of odds) are very convenient but one must keep in mind that the odds against χ^2 as a measure of correlation (not goodness of fit) may be very much greater.

Relation to percentage crossing over

For theoretical distributions there is fortunately a very simple relationship between the coefficient of correlation of zygotic phenotypes and the crossing over percentage of the two genetic factors under consideration. In the male the frequency of the AB or ab gametes may be represented by p and the Ab or aB gametes by $(1-p)$ if conditions are normal. Likewise in the female let p' represent the AB or ab gametes and $(1-p')$ the Ab or aB gametes. This leads to the following comparison in the F_2 generation.

AB	Ab	aB	ab
$2+pp'$	$1-pp'$	$1-pp'$	pp'

If one can further assume that the crossing over in the female has been the same as that in the male the problem becomes still more simple, that is, $pp'=p^2$.

Now calculating the coefficient of correlation from formula [1]

$$r=\frac{4p^2-1}{3} \text{ or } p=\frac{\sqrt{3r+1}}{2}$$

With similar assumptions (that $p=p'$ etc.) it is possible to estimate the crossing over percentage from observed data for various factorial relationships.

The modification of formula [1], suggested by KELLEY (1924, page 259) is preferable for this purpose. From the definitions

$$p_1=\frac{a+b}{n}, \qquad q_1=\frac{c+d}{n}, \qquad p_2=\frac{a+c}{n} \text{ and } q_2=\frac{b+d}{n}$$

$$r=\frac{d-q_1q_2}{\sqrt{p_1p_2q_1q_2}} \tag{4}$$

The simplicity of this formula should be appreciated by students of genetics because it brings out the relationship between the coefficient of correlation and percentage crossing over for various complex sorts of Mendelian combinations. Furthermore it illustrates a convenient method of building up a theoretical distribution when only the phenotypic ratios and the coefficient of correlation are known, because

$$d = r\sqrt{p_1 p_2 q_1 q_2} + q_1 q_2 \qquad (5)$$

and since $q_1 = \frac{c+d}{n}$, etc., c, b and d can be calculated very readily.

Table 1 gives the values of p (proportion of AB or ab gametes) in terms of r for the Mendelian combinations which are most likely to be considered. The less practical formulae, (COLLINS 1924) calculated directly according to EMERSON'S (1916) original method, are also included.

TABLE 1

Crossing over percentage from coefficient of correlation compared with direct calculation.

THEORETICAL PHENOTYPIC RATIOS	VALUE OF p^2 FROM DIRECT CALCULATION	VALUE OF p^2 FROM COEFFICIENT OF CORRELATION
1:1 and 1:1 (Backcross)	$\left(\frac{a+d}{n}\right)^2$	$\frac{(r+1)^2}{4}$
3:1 and 3:1 (No crossing over in one sex)[1]	$\left(\frac{2(a+d)}{n}-1\right)^2$	$\frac{(3r+1)^2}{16}$
3:1 and 3:1	$\frac{2(a+d)}{n}-1$	$\frac{3r+1}{4}$
15:1 and 3:1	$\frac{8(a+d)}{n}-5.5$	$\frac{3\sqrt{5}r+1}{4}$
63:1 and 3:1	$\frac{32(a+d)}{n}-23.5$	$\frac{3\sqrt{21}r+1}{4}$
15:1 and 15:1	$\frac{32(a+d)}{n}-28$	$\frac{15r+1}{4}$
9:7 and 3:1	$\frac{16(a+d)-7n}{6n}$	$\frac{\sqrt{21}r+1}{4}$
27:37 and 3:1	$\frac{64(a+d)-25n}{18n}$	$\frac{\sqrt{37}r+1}{4}$
9:7 and 9:7	$\frac{32(a+d)-14n}{9n}$	$\frac{7r+1}{4}$
9:7 and 15:1	$\frac{32(a+d)-17n}{3n}$	$\frac{\sqrt{105}r+1}{4}$

[1] All other formulae are based on equal crossing over percentages in both sexes.

As a practical procedure r may be calculated from an observed distribution if the characters are clear-cut. If the ratios are abnormal, however, it is obvious that a value of p for a theoretical distribution corresponding to this value of r may not be the true value of p, but it should be a very good approximation. In any event it represents the same amount of

correlation in a theoretical distribution that is implied by the value of r derived from the observed distribution. It is doubtful if a better assumption can be made unless there is knowledge of some abnormality. Furthermore the value of r is conservative and for all the abnormal deviations due to differential mortality of gametes or zygotes calculated in tables 2, 3 and 4, r does not indicate more linkage than that implied by the *a priori* crossover values.

COMPARISON OF VARIOUS METHODS OF ESTIMATING LINKAGE VALUES

The methods of estimating linkage values may be grouped into two general classes, 1st, those which make direct comparisons with theoretical ratios—FISHER'S, HALDANE'S and EMERSON'S. 2nd, those which make comparisons with observed ratios—YULE'S coefficient of association Q and the coefficient of correlation r[1].

FISHER'S and HALDANE'S methods give practically identical results as should be expected because they are based upon the same principles but the former is more simply applied. For theoretical ratios EMERSON'S method gives the same results as all other methods but it is not well adapted to practical use where slight deviations from the theoretical results are of common occurrence.

The coefficient of correlation r is a more stable and reliable measure of correlation than the coefficient of association Q and is therefore more adaptable for general use. However, Q is especially useful when there is differential death rate of zygotes (See also COLLINS 1924) and should probably be calculated simultaneously with r as a general practise. In fact this is especially convenient because of the similarity of the two formulae, [1] and [7].

For a comparison of these five methods see tables 2, 3 and 4 which were first suggested by COLLINS (1924).

Fisher's method

FISHER (1925, page 25) has called attention to a very ingenious method of deriving a theoretical F_2 distribution which gives the best fit in comparison with the observed distribution. It is a simple method with all the good qualities of HALDANE'S T (1919) and may well replace the original formula suggested by EMERSON (1916).

FISHER'S method is based upon the value of p which makes χ^2 a minimum. This is readily accomplished by multiplying the observed frequen-

[1] Termed the Boas-Yulean ϕ by PEARSON and HERON (1913).

cies by the logarithm of their theoretical functions and finding the value of p which makes the sum of these products a maximum. The necessary differentiation and algebraic transformation makes it possible to write

$$p^2 = \frac{(a-2b-2c-d)+\sqrt{(a-2b-2c-d)^2+8dn}}{2n} \qquad (6)$$

for an F_2 distribution (with 3:1 and 3:1 ratios). If there is no crossing over in one sex a similar procedure gives

$$p = \frac{(a-2b-2c-d)+\sqrt{(a-2b-2c-d)^2+8dn}}{2n}$$

and for the simple case of backcrossing (with 1:1 and 1:1 ratios)

TABLE 2

Comparison of methods of measuring linkage values in a backcross— (1:1 and 1:1 ratios).

	ZYGOTIC CLASSES—IN PERCENT				OBSERVED VALUES OF p^1 FROM:		
	a	b	c	d	EMERSON'S[2] p	YULE'S Q	r
No actual gametic linkage							
Perfect population	25.00	25.00	25.00	25.00	50.00	50.00	50.00
25 percent a gametes not effective	28.57	28.57	21.43	21.43	50.00	50.00	50.00
25 percent a and b gametes not effective	32.65	24.49	24.49	18.37	51.02	50.00	50.00
25 percent a and B gametes not effective	24.49	32.65	18.37	24.49	48.98	50.00	50.00
25 percent a zygotes wrongly classified as A	31.25	31.25	18.75	18.75	50.00	50.00	50.00
25 percent actual gametic linkage—Coupling phase[1]							
Perfect population	37.50	12.50	12.50	37.50	75.00	75.00	75.00
25 percent a gametes not effective	42.86	14.29	10.71	32.14	75.00	75.00	74.81
25 percent a and b gametes not effective	48.48	12.12	12.12	27.27	75.77	75.00	74.61
25 percent a and B gametes not effective	37.11	9.28	16.49	37.11	74.24	75.00	74.61
25 percent a zygotes wrongly classified as A	40.63	21.88	9.38	28.13	68.75	79.76	69.37

[1] For coupling phase the crossing over percentage is equal to $1-p$.

[2] EMERSON'S direct method gives the closest fit to the theoretical ratios for backcrosses and may, therefore, be considered synonymous with FISHER'S and HALDANE'S methods.

$$p = \frac{a+d}{n}$$

the direct value which has been customarily used.

More complex relations may also be treated in a similar manner. Unfortunately, it is necessary to solve a cubic equation unless two of the theoretical classes are identical.

As long as the observed distribution can be thought of as a normal deviation from the theoretical, there is good justification for FISHER'S method and a crossing over percentage calculated in this way is a very good approximation to that which really takes place. However, it may frequently be desirable to know the actual correlation as indicated by the coefficient of correlation because linkage (or correlation) may be implied by FISHER'S method when actually none exists (tables 2, 3 and 4.) See COLLINS, 1924, for a similar criticism of HALDANE'S method.

TABLE 3

Comparison of methods of measuring linkage values in F_2—no actual gametic linkage.

	ZYGOTIC CLASSES—IN PERCENT				OBSERVED VALUES OF p FROM:			
	a	b	c	d	EMERSON'S p	YULE'S Q	FISHER'S OR HALDANE'S p	r
Perfect population	56.25	18.75	18.75	6.25	50.00	50.00	50.00	50.00
25 percent a gametes not effective	61.22	20.41	13.78	4.59	56.24	50.00	50.00	50.00
25 percent a and b gametes not effective	66.64	14.99	14.99	3.37	63.26	50.00	52.58	50.00
25 percent a and B gametes not effective	54.98	26.66	12.37	6.00	46.85	50.00	47.97	50.00
25 percent a zygotes die	60.00	20.00	15.00	5.00	54.77	50.00	50.00	50.00
25 percent a and b zygotes die	64.00	16.00	16.00	4.00	60.00	50.00	51.33	50.00
25 percent a and B zygotes die	55.38	24.62	13.85	6.15	48.04	50.00	48.83	50.00
25 percent a zygotes called A	60.94	20.31	14.06	4.69	55.90	50.00	50.00	50.00
Double recessive class not recovered	60.00	20.00	20.00	0.00	44.72	0.00	0.00	25.00

TABLE 4

Comparison of methods of measuring linkage values in F_2—25 percent actual gametic linkage.

	ZYGOTIC CLASSES—IN PERCENT				OBSERVED VALUES OF p FROM:			
	a	*b*	*c*	*d*	EMERSON'S p	YULE'S Q	FISHER'S OR HALDANE'S p	r
Coupling phase								
Perfect population	64.06	10.94	10.94	14.06	75.00	75.00	75.00	75.00
25 percent *a* gametes not effective	70.41	11.22	8.04	10.33	78.41	74.32	75.82	74.17
25 percent *a* and *b* gametes not effective	76.40	8.08	8.08	7.44	82.26	73.59	78.11	73.33
25 percent *a* and *B* gametes not effective	63.51	14.96	7.75	13.77	73.88	74.98	73.95	74.56
25 percent *a* zygotes die	68.33	11.67	8.75	11.25	76.92	75.00	75.00	73.98
25 percent *a* and *b* zygotes die	72.49	9.28	9.28	8.95	79.29	75.00	75.85	73.01
25 percent *a* and *B* zygotes die	63.48	14.45	8.13	13.94	74.05	75.00	74.18	74.68
25 percent *a* zygotes called *A*	66.80	14.45	8.20	10.55	73.95	77.42	71.90	71.41
One crossover class not recovered	71.93	12.28	0.00	15.78	86.84	100.00	86.12	87.74
Repulsion phase								
Perfect population	51.56	23.44	23.44	1.56	25.00	25.00	25.00	25.00
25 percent *a* gametes not effective	54.06	27.55	17.22	1.15	32.34	23.77	23.35	27.10
25 percent *a* and *b* gametes not effective	57.82	20.66	20.66	0.86	41.67	22.63	23.48	28.74
25 percent *a* and *B* gametes not effective	49.22	35.26	14.05	1.47	11.74	24.96	22.87	26.11
25 percent *a* zygotes die	55.00	25.00	18.75	1.25	35.36	25.00	25.00	28.20
25 percent *a* and *b* zygotes die	58.86	20.07	20.07	1.00	44.42	25.00	26.18	30.83
25 percent *a* and *B* zygotes die	50.57	30.65	17.24	1.53	20.53	25.00	24.14	25.41
25 percent *a* zygotes called *A*	57.42	23.83	17.58	1.17	41.46	26.00	26.40	30.65
Double recessive not recovered	52.38	23.81	23.81	0.00	21.82	0.00	0.00	12.50

Yule's coefficient of association Q

The coefficient of association Q has been a source of much controversy as a measure of actual correlation (YULE 1912 and PEARSON and HERON 1913). The disadvantages of this method, as a general method, are well appreciated and the reader is referred to the discussion given by PEARSON

and HERON. However, the defects in the method as a measure of correlation really become an asset in estimating crossing over percentages in certain instances. Its use in connection with the coefficient of correlation may, therefore, be highly recommended, but certainly indiscriminate usage (as feared by PEARSON and HERON) should by all means be avoided.

The formula under discussion is

$$Q=\frac{ad-bc}{ad+bc} \tag{7}$$

The use of this formula is limited to discrete units the same as the coefficient of correlation. But granting this assumption it has some very peculiar properties which must be known before it can be properly employed.

First it should be pointed out (see illustrations in tables 2, 3 and 4) that Q represents perfect negative correlation when $d=0$ and perfect positive correlation when either $b=0$ or when $c=0$. It is very obvious that this can not be true.

PEARSON and HERON have shown that a fourfold distribution may be manipulated so that r will remain constant but Q will rise at will and with Q constant r can fall to zero.

These properties clearly show the limitations of Q but certain advantages arise from the following property.

The value of Q is not changed by multiplying $(a+b)$, $(c+d)$, $(a+c)$, or $(c+d)$ by any factor because the fourfold table

ka	b	ka+b
kc	d	kc+d
k(a+c)	(b+d)	n

gives the same value for Q for all values of k. This property is certainly a very decided advantage in the case of differential death rate of zygotes due to one of the factors under consideration acting as a lethal or semi-lethal factor.

For specific ratios there is a definite relation between r and Q. YULE (1900) gives the following general relationship:

$$k=\frac{1-Q}{1+Q} \qquad r=\frac{\cos\sqrt{k}}{1+\sqrt{k}}\pi$$

For the Mendelian ratios (3:1 and 3:1)

$$Q=\frac{24r}{(3r+1)^2+8}$$

If Q is to be used as a measure of correlation it is also very necessary to consider its probable error.

$$E_Q=.6745\times\frac{1-Q^2}{2}\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}} \tag{8}$$

because this may be different (PEARSON and HERON 1913, page 175) for the same values of Q and n. It will be noted that this formula may be simpler than that for coefficient of correlation (see formula [3]).

Unfortunately Q is not so well adapted to algebraic manipulation as the coefficient of correlation r but a determination of p from corresponding values of Q can be made by a relatively small amount of labor.

In an F_2 distribution (3:1 and 3:1 ratios),[1]

$$p^2=\frac{2-\sqrt{4-2Q-2Q^2}}{2Q} \tag{9}$$

For an F_2 distribution with complete linkage in one sex

$$p=\frac{2-\sqrt{4-2Q-2Q^2}}{2Q} \tag{10}$$

and for backcrossing (1:1 and 1:1 ratios).

$$p=\frac{Q+1-\sqrt{1-Q^2}}{2Q} \tag{11}$$

Further combinations may be calculated in a similar manner from the theoretical values of a'', b'' c'' and d'', in terms of p. (See tables 5 to 15).

Examples

Example 1—(3:1 and 3:1 ratios). This example is taken from an experiment with maize by EAST and HAYES presented by BABCOCK and CLAUSEN (1918).

The observed data compared with a 9:3:3:1 distribution are as follows:

Purple, starchy	*Purple, sweet*	*White, starchy*	*White, sweet*
1,861	614	548	217
1,822.5	607.5	607.5	202.5

[1] When $Q=0$ it is necessary to differentiate both numerator and denominator to get the proper value for p.

A comparison with theoretical ratios without correlation was considered which led to $\chi^2=7.75$ and the odds against a fit as bad as this were found to be about 18:1 from ELDERTON'S tables. From a consideration of χ^2 as a measure of correlation a value of $\chi^2=7.75$ would probably be very significant but by making the comparison with theoretical ratios one cannot easily see whether the deviation from a perfect fit is due mostly to aberrations in ratios or to linkage. The preferable calculation from formula [2] making χ^2 a measure of correlation gives: $\chi^2=3.88$. It will be noted that this is considerably less than the value derived from the previous method showing that correlation (or linkage) alone was not the only cause of the relatively poor fit.

The coefficient of correlation from formula [1] is: $r=0.0346$. The standard deviation of r considering random distribution is $\frac{1}{\sqrt{n}}$ or 0.0176. Dividing r by ${}_0\sigma_r$ is the same as taking the square root of χ^2. This gives $\chi=1.97$. This value may be slightly suspicious but can hardly be considered significant, but if χ^2 (due to correlation) had been 7.75, then $\frac{r}{{}_0\sigma_r}$ would have been 2.78[1], a much more significant figure.

The value of p (proportion of AB or ab gametes) may be calculated in four different ways for this particular problem without marked differences. These are as follows:

Method	*Value of p in percent*
r	52.53
Q	52.56
FISHER'S p	52.52
EMERSON'S p	53.17

In this example the deficiency is largely in the white, starchy class. This causes the ratios to deviate considerably from the theoretical but not in such a way as to indicate differential zygotic death rates due to either of the factors under consideration. Differential mortality due to the particular combination of factors would be a more logical expectation from the data available.

If the correlation is not significant and if larger F_2 populations were grown the crossing over percentage would be expected to approach 50 percent more closely. However, if increased numbers still indicated a value for p of 0.525 it would finally become significant. Then the crossing over

[1] If the frequency of r in terms of ${}_0\sigma_r$ were Gaussian a deviation of 2.78 times ${}_0\sigma_r$ would represent odds of 183:1. See text for a discussion of this subject. See also PEARSON'S table XV(C) (1914).

percentage would be $(1-p)\times 100$ or 47.5 percent. A comparison with theoretical ratios may be seen in tables 7 and 8.

Example 2—(15:1 and 3:1 ratios.)

This example is taken from data published by the writer (OWEN 1927, progeny no. 13). Very similar observations were also made by WOODWORTH (1923). Duplicate factors, D_1 and D_2, are assumed to be equally capable of producing yellow cotyledon color. The factor D_1 is apparently linked with G, the factor for green seed-coat color. The recessive characters are green-cotyledon color and yellow seed-coat color respectively. The observed F_2 data compared with a theoretical random distribution are as follows:

	YELLOW COTYLEDONS		GREEN COTYLEDONS	
	Green seed-coat	Yellow seed-coat	Green seed-coat	Yellow seed-coat
Observed	236	85	16	0
Calculated	*236.9*	*79.0*	*15.8*	*5.3*

Calculating χ^2 in the usual way by comparing the observed distribution with a theoretical distribution with 15:1 and 3:1 ratios and 50 percent crossing over gives $\chi^2=5.72$. If the data are corrected to give an exact 15:1 ratio for cotyledon color which seemed logical because of the necessity of growing F_2 seed to determine the color of the F_2 seed coats, another calculation gives $\chi^2=5.26$. It makes little difference which value is considered, that is, $\chi^2=5.27$ or 5.26. From ELDERTON'S tables the odds against a fit as bad as the observed due to chance alone are approximately 8:1. Is this significant?

In contrast with the conclusions that might be drawn from the above paragraph consider the value of χ^2 from formula [2]. This is 7.6 and the odds from ELDERTON'S tables are nearly 20:1. Is this significant? Surely much more suspicion is likely to arise than from the 8:1 odds but one may be somewhat puzzled until he realizes that this value of χ^2 is a measure of correlation. It was previously shown that the probability of securing the double recessive class in 337 observations threw more light on the subject. The probability of securing 16 F_2 seeds with green cotyledons without a single F_2 plant (of the 16) having yellow seed coats leads to a very definite conclusion. The odds against random distribution are $1:(3/4)^{16}$ or approximately 100:1 and additional information gained from subsequent F_2 populations continued to indicate a significant correlation.

If the formulae given in table 1 are used to estimate the crossing over percentage the direct calculation gives 32.00 percent while the calculation from r gives 18.04 percent. Previously the writer found by inspection (see table 10) that approximately 13 percent crossing over gave a very good comparison with the observed distribution. This illustrates the usefulness of the coefficient of correlation because the direct calculation (which gave 32 percent) is very inaccurate especially in the repulsion phase. In this particular problem the coefficient of association fails entirely to express a logical crossover value. From formula [7] $Q=-1.00$ which indicates complete repulsion and would imply that D_1 and g were completely linked.

SUMMARY

A method has been suggested for calculating linkage intensities by means of a product moment coefficient of correlation r. This is recommended as a general method in preference to the other methods with which it has been compared.

YULE'S coefficient of association Q is a valuable supplement to the coefficient of correlation, and when there is knowledge of differential mortality of zygotes it gives a better approximation to the true crossing over percentage.

FISHER'S method has also been found very convenient but its use is limited to distributions where observed ratios closely approach the theoretical ratios.

Tables similar to those given by COLLINS (1924) have been used to illustrate the various methods.

Much value is attached to the coefficient of correlation because it may be readily applied to complex relationships as well as to simple cases of backcrossing. Formulae for the most general cases are given and tables for theoretical distributions are included in the appendix.

The convenient relationship between χ^2 and r has been illustrated. When χ^2 is a measure of correlation, $\chi^2=nr^2$ and $\sqrt{n}\,r^2=\chi=\frac{r}{{}_o\sigma_r}$.

Attention has been called to the fact that r and Q, for accurate estimates, apply only to distributions made up of discrete units where the characters are clear-cut. Otherwise an error may be introduced which is not easily measured.

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APPENDIX

TABLE 5

Crossing over percentages and values of r and Q for an F_1 backcrossed with the double recessive—1:1 and 1:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				Q	r
	a''	b''	c''	d''		
	$\frac{p}{2}$	$\frac{1-p}{2}$	$\frac{1-p}{2}$	$\frac{p}{2}$	$\frac{2p-1}{2p^2-2p+1}$	$\frac{2p-1}{1}$
Coupling phase						
0	50.00	0.00	0.00	50.00	1.0000	1.00
5	47.50	2.50	2.50	47.50	0.9945	0.90
10	45.00	5.00	5.00	45.00	0.9757	0.80
15	42.50	7.50	7.50	42.50	0.9396	0.70
20	40.00	10.00	10.00	40.00	0.8824	0.60
25	37.50	12.50	12.50	37.50	0.8000	0.50
30	35.00	15.00	15.00	35.00	0.6897	0.40
35	32.50	17.50	17.50	32.50	0.5505	0.30
40	30.00	20.00	20.00	30.00	0.3846	0.20
45	27.50	22.50	22.50	27.50	0.1980	0.10
50	25.00	25.00	25.00	25.00	0.0000	0.00
Repulsion phase						
0	0.00	50.00	50.00	0.00	−1.0000	−1.00
5	2.50	47.50	47.50	2.50	−0.9945	−0.90
10	5.00	45.00	45.00	5.00	−0.9757	−0.80
15	7.50	42.50	42.50	7.50	−0.9396	−0.70
20	10.00	40.00	40.00	10.00	−0.8824	−0.60
25	12.50	37.50	37.50	12.50	−0.8000	−0.50
30	15.00	35.00	35.00	15.00	−0.6897	−0.40
35	17.50	32.50	32.50	17.50	−0.5505	−0.30
40	20.00	30.00	30.00	20.00	−0.3846	−0.20
45	22.50	27.50	27.50	22.50	−0.1980	−0.10
50	25.00	25.00	25.00	25.00	0.0000	0.00

TABLE 6

Crossing over percentages when there is complete linkage in one sex—3:1 and 3:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—PERCENT				Q	r
	a''	b''	c''	d''		
	$\frac{2+p}{4}$	$\frac{1-p}{4}$	$\frac{1-p}{4}$	$\frac{p}{4}$	$\frac{4p-1}{2p^2+1}$	$\frac{4p-1}{3}$
			Coupling phase			
0	75.00	0.00	0.00	25.00	1.0000	1.0000
5	73.75	1.25	1.25	23.75	0.9982	0.9333
10	72.50	2.50	2.50	22.50	0.9923	0.8667
15	71.25	3.75	3.75	21.25	0.9816	0.8000
20	70.00	5.00	5.00	20.00	0.9649	0.7333
25	68.75	6.25	6.25	18.75	0.9412	0.6667
30	67.50	7.50	7.50	17.50	0.9091	0.6000
35	66.25	8.75	8.75	16.25	0.8672	0.5333
40	65.00	10.00	10.00	15.00	0.8140	0.4667
45	63.75	11.25	11.25	13.75	0.7476	0.4000
50	62.50	12.50	12.50	12.50	0.6667	0.3333
			Repulsion phase			
0	50.00	25.00	25.00	0.00	−1.0000	−0.3333
5	51.25	23.75	23.75	1.25	−0.7960	−0.2667
10	52.50	22.50	22.50	2.50	−0.5882	−0.2000
15	53.75	21.25	21.25	3.75	−0.3828	−0.1333
20	55.00	20.00	20.00	5.00	−0.1852	−0.0667
25	56.25	18.75	18.75	6.25	0.0000	0.0000
30	57.50	17.50	17.50	7.50	0.1695	0.0667
35	58.75	16.25	16.25	8.75	0.3213	0.1333
40	60.00	15.00	15.00	10.00	0.4545	0.2000
45	61.25	13.75	13.75	11.25	0.5694	0.2667
50	62.50	12.50	12.50	12.50	0.6667	0.3333

TABLE 7

Crossing over percentages and values of Q and r with corresponding theoretical F_2 phenotypic ratios with single factors for both characters—3:1 and 3:1 ratios—Coupling phase.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				Q	r
	a''	b''	c''	d''	COEFFICIENT OF ASSOCIATION	COEFFICIENT OF CORRELATION
	$\frac{2+p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{p^2}{4}$	$\frac{4p^2-1}{2p^4+1}$	$\frac{4p^2-1}{3}$
0	75.00	0.00	0.00	25.00	1.0000	1.0000
1	74.50	0.50	0.50	24.50	0.9997	0.9735
2	74.01	0.99	0.99	24.01	0.9989	0.9472
3	73.52	1.48	1.48	23.52	0.9975	0.9212
4	73.04	1.96	1.96	23.04	0.9954	0.8955
5	72.56	2.44	2.44	22.56	0.9928	0.8700
6	72.09	2.91	2.91	22.09	0.9894	0.8448
7	71.62	3.38	3.38	21.62	0.9854	0.8199
8	71.16	3.84	3.84	21.16	0.9806	0.7952
9	70.70	4.30	4.30	20.70	0.9751	0.7708
10	70.25	4.75	4.75	20.25	0.9688	0.7467
11	69.80	5.20	5.20	19.80	0.9617	0.7228
12	69.36	5.64	5.64	19.36	0.9537	0.6992
13	68.92	6.08	6.08	18.92	0.9449	0.6759
14	68.49	6.51	6.51	18.49	0.9352	0.6528
15	68.06	6.94	6.94	18.06	0.9247	0.6300
16	67.64	7.36	7.36	17.64	0.9131	0.6075
17	67.22	7.78	7.78	17.22	0.9007	0.5852
18	66.81	8.19	8.19	16.81	0.8873	0.5632
19	66.40	8.60	8.60	16.40	0.8729	0.5415
20	66.00	9.00	9.00	16.00	0.8575	0.5200
21	65.60	9.40	9.40	15.60	0.8411	0.4988
22	65.21	9.79	9.79	15.21	0.8238	0.4779
23	64.82	10.18	10.18	14.82	0.8054	0.4572
24	64.44	10.56	10.56	14.44	0.7860	0.4368
25	64.06	10.94	10.94	14.06	0.7656	0.4167
26	63.69	11.31	11.31	13.69	0.7441	0.3968
27	63.32	11.68	11.68	13.32	0.7217	0.3772
28	62.96	12.04	12.04	12.96	0.6983	0.3579
29	62.60	12.40	12.40	12.60	0.6739	0.3388
30	62.25	12.75	12.75	12.25	0.6486	0.3200
31	61.90	13.10	13.10	11.90	0.6223	0.3015
32	61.56	13.44	13.44	11.56	0.5951	0.2832
33	61.22	13.78	13.78	11.22	0.5671	0.2652
34	60.89	14.11	14.11	10.89	0.5382	0.2475
35	60.56	14.44	14.44	10.56	0.5085	0.2300
36	60.24	14.76	14.76	10.24	0.4780	0.2128
37	59.92	15.08	15.08	9.92	0.4468	0.1959
38	59.61	15.39	15.39	9.61	0.4150	0.1792
39	59.30	15.70	15.70	9.30	0.3825	0.1628

TABLE 7—continued

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				Q	r
	a''	b''	c''	d''	COEFFICIENT OF ASSOCIATION	COEFFICIENT OF CORRELATION
	$\frac{2+p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{p^2}{4}$	$\frac{4p^2-1}{2p^4+1}$	$\frac{4p^2-1}{3}$
40	59.00	16.00	16.00	9.00	0.3494	0.1467
41	58.70	16.30	16.30	8.70	0.3159	0.1308
42	58.41	16.59	16.59	8.41	0.2818	0.1152
43	58.12	16.88	16.88	8.12	0.2474	0.0999
44	57.84	17.16	17.16	7.84	0.2126	0.0848
45	57.56	17.44	17.44	7.56	0.1775	0.0700
46	57.29	17.71	17.71	7.29	0.1422	0.0555
47	57.02	17.98	17.98	7.02	0.1068	0.0412
48	56.76	18.24	18.24	6.76	0.0712	0.0272
49	56.50	18.50	18.50	6.50	0.0356	0.0135
50	56.25	18.75	18.75	6.25	0.0000	0.0000

TABLE 8

Crossing over percentages and values of Q and r with corresponding theoretical F_2 phenotypic ratios with single factors for both characters—3:1 and 3:1 ratios—Repulsion phase.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				Q	r
	a''	b''	c''	d''	COEFFICIENT OF ASSOCIATION	COEFFICIENT OF CORRELATION
	$\frac{2+p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{p^2}{4}$	$\frac{4p^2-1}{2p^4+1}$	$\frac{4p^2-1}{3}$
0	50.00	25.00	25.00	0.00	−1.0000	−0.3333
1	50.00	25.00	25.00	0.00	−0.9996	−0.3332
2	50.01	24.99	24.99	0.01	−0.9984	−0.3328
3	50.02	24.98	24.98	0.02	−0.9964	−0.3321
4	50.04	24.96	24.96	0.04	−0.9936	−0.3312
5	50.06	24.94	24.94	0.06	−0.9900	−0.3300
6	50.09	24.91	24.91	0.09	−0.9856	−0.3285
7	50.12	24.88	24.88	0.12	−0.9804	−0.3268
8	50.16	24.84	24.84	0.16	−0.9743	−0.3248
9	50.20	24.80	24.80	0.20	−0.9675	−0.3225
10	50.25	24.75	24.75	0.25	−0.9598	−0.3200
11	50.30	24.70	24.70	0.30	−0.9513	−0.3172
12	50.36	24.64	24.64	0.36	−0.9420	−0.3141
13	50.42	24.58	24.58	0.42	−0.9319	−0.3108
14	50.49	24.51	24.51	0.49	−0.9209	−0.3072
15	50.56	24.44	24.44	0.56	−0.9091	−0.3033
16	50.64	24.36	24.36	0.64	−0.8964	−0.2992

TABLE 8—continued

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				Q	r
	a''	b''	c''	d''	COEFFICIENT OF ASSOCIATION	COEFFICIENT OF CORRELATION
	$\frac{2+p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{1-p^2}{4}$	$\frac{p^2}{4}$	$\frac{4p^2-1}{2p^4+1}$	$\frac{4p^2-1}{3}$
17	50.72	24.28	24.28	0.72	−0.8829	−0.2948
18	50.81	24.19	24.19	0.81	−0.8686	−0.2901
19	50.90	24.10	24.10	0.90	−0.8534	−0.2852
20	51.00	24.00	24.00	1.00	−0.8373	−0.2800
21	51.10	23.90	23.90	1.10	−0.8204	−0.2745
22	51.21	23.79	23.79	1.21	−0.8026	−0.2688
23	51.32	23.68	23.68	1.32	−0.7840	−0.2628
24	51.44	23.56	23.56	1.44	−0.7645	−0.2565
25	51.56	23.44	23.44	1.56	−0.7442	−0.2500
26	51.69	23.31	23.31	1.69	−0.7230	−0.2432
27	51.82	23.18	23.18	1.82	−0.7009	−0.2361
28	51.96	23.04	23.04	1.96	−0.6781	−0.2288
29	52.10	22.90	22.90	2.10	−0.6543	−0.2212
30	52.25	22.75	22.75	2.25	−0.6298	−0.2133
31	52.40	22.60	22.60	2.40	−0.6044	−0.2052
32	52.56	22.44	22.44	2.56	−0.5783	−0.1968
33	52.72	22.28	22.28	2.72	−0.5513	−0.1881
34	52.89	22.11	22.11	2.89	−0.5236	−0.1792
35	53.06	21.94	21.94	3.06	−0.4951	−0.1700
36	53.24	21.76	21.76	3.24	−0.4659	−0.1605
37	53.42	21.58	21.58	3.42	−0.4361	−0.1508
38	53.61	21.39	21.39	3.61	−0.4055	−0.1408
39	53.80	21.20	21.20	3.80	−0.3743	−0.1305
40	54.00	21.00	21.00	4.00	−0.3425	−0.1200
41	54.20	20.80	20.80	4.20	−0.3101	−0.1092
42	54.41	20.59	20.59	4.41	−0.2772	−0.0981
43	54.62	20.38	20.38	4.62	−0.2437	−0.0868
44	54.84	20.16	20.16	4.84	−0.2099	−0.0752
45	55.06	19.94	19.94	5.06	−0.1756	−0.0633
46	55.29	19.71	19.71	5.29	−0.1410	−0.0512
47	55.52	19.48	19.48	5.52	−0.1061	−0.0388
48	55.76	19.24	19.24	5.76	−0.0709	−0.0261
49	56.00	19.00	19.00	6.00	−0.0355	−0.0132
50	56.25	18.75	18.75	6.25	0.0000	0.0000

TABLE 9

Crossing over percentages and values of r when one character depends upon complementary factors—9:7 and 3:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				r
	a''	b''	c''	d''	
	$\frac{6+3p^2}{16}$	$\frac{3-3p^2}{16}$	$\frac{6-3p^2}{16}$	$\frac{1+3p^2}{16}$	$\frac{4p^2-1}{\sqrt{21}}$
Coupling phase					
0	56.25	0.00	18.75	25.00	0.6547
5	54.42	1.83	20.58	23.17	0.5695
10	52.69	3.56	22.31	21.44	0.4888
15	51.05	5.20	23.95	19.80	0.4124
20	49.50	6.75	25.50	18.25	0.3404
25	48.05	8.20	26.95	16.80	0.2728
30	46.69	9.56	28.31	15.44	0.2095
35	45.42	10.83	29.58	14.17	0.1506
40	44.25	12.00	30.75	13.00	0.0960
45	43.17	13.08	31.83	11.92	0.0458
50	42.19	14.06	32.81	10.94	0.0000
Repulsion phase					
0	37.50	18.75	37.50	6.25	−0.2182
5	37.55	18.70	37.45	6.30	−0.2160
10	37.69	18.56	37.31	6.44	−0.2095
15	37.92	18.33	37.08	6.67	−0.1986
20	38.25	18.63	36.75	7.00	−0.1833
25	38.67	17.58	36.33	7.42	−0.1637
30	39.19	17.06	35.81	7.94	−0.1397
35	39.80	16.45	35.20	8.55	−0.1113
40	40.50	15.75	34.50	9.25	−0.0786
45	41.30	14.95	33.70	10.05	−0.0415
50	42.19	14.06	32.81	10.94	0.0000

TABLE 10

Crossing over percentages and values of r when one character is determined by duplicate factors—15:1 and 3:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				r
	a''	b''	c''	d''	
	$\frac{11+p^2}{16}$	$\frac{4-p^2}{16}$	$\frac{1-p^2}{16}$	$\frac{p^2}{16}$	$\frac{4p^2-1}{3\sqrt{5}}$
Coupling phase					
0	75.00	18.75	0.00	6.25	0.4472
5	74.39	19.36	0.61	5.64	0.3891
10	73.81	19.94	1.19	5.06	0.3339
15	73.27	20.48	1.73	4.52	0.2817
20	72.75	21.00	2.25	4.00	0.2326
25	72.27	21.48	2.73	3.52	0.1863
30	71.81	21.94	3.19	3.06	0.1431
35	71.39	22.36	3.61	2.64	0.1029
40	71.00	22.75	4.00	2.25	0.0656
45	70.64	23.11	4.36	1.89	0.0313
50	70.31	23.44	4.69	1.56	0.0000
Repulsion phase					
0	68.75	25.00	6.25	0.00	−0.1491
5	68.77	24.98	6.23	0.02	−0.1476
10	68.81	24.94	6.19	0.06	−0.1431
15	68.89	24.86	6.11	0.14	−0.1357
20	69.00	24.75	6.00	0.25	−0.1252
25	69.14	24.61	5.86	0.39	−0.1118
30	69.31	24.44	5.69	0.56	−0.0954
35	69.52	24.23	5.48	0.77	−0.0760
40	69.75	24.00	5.25	1.00	−0.0537
45	70.02	23.74	4.98	1.27	−0.0283
50	70.31	23.44	4.69	1.56	0.0000

TABLE 11

Crossing over percentages and values of r when one character depends upon three supplementary factors—27:37 and 3:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				
	a''	b''	c''	d''	r
	$\frac{18+9p^2}{64}$	$\frac{9-9p^2}{64}$	$\frac{30-9p^2}{64}$	$\frac{7+9p^2}{64}$	$\frac{4p^2-1}{\sqrt{37}}$
Coupling phase					
0	42.19	0.00	32.81	25.00	0.4932
5	40.82	1.37	34.18	23.63	0.4291
10	39.52	2.67	35.48	22.33	0.3683
15	38.29	3.90	36.71	21.10	0.3107
20	37.13	5.06	37.87	19.94	0.2565
25	36.04	6.15	38.96	18.85	0.2055
30	35.02	7.17	39.98	17.83	0.1578
35	34.07	8.12	40.93	16.88	0.1134
40	33.19	9.00	41.81	16.00	0.0723
45	32.38	9.81	42.62	15.19	0.0345
50	31.64	10.55	43.36	14.45	0.0000
Repulsion phase					
0	28.13	14.06	46.87	10.94	−0.1644
5	28.16	14.03	46.84	10.97	−0.1628
10	28.27	13.92	46.73	11.08	−0.1578
15	28.44	13.75	46.56	11.25	−0.1496
20	28.69	13.50	46.31	11.50	−0.1381
25	29.00	13.18	46.00	11.82	−0.1233
30	29.39	12.80	45.61	12.20	−0.1052
35	29.85	12.34	45.15	12.66	−0.0838
40	30.37	11.81	44.63	13.19	−0.0592
45	30.97	11.21	44.03	13.79	−0.0312
50	31.64	10.55	43.36	14.45	0.0000

TABLE 12

Crossing over percentages when one character is determined by triplicate factors—63:1 and 3:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				r
	a''	b''	c''	d''	
	$\frac{47+p^2}{64}$	$\frac{16-p^2}{64}$	$\frac{1-p^2}{64}$	$\frac{p^2}{64}$	$\frac{4p^2-1}{3\sqrt{21}}$
Coupling phase					
0	75.00	23.44	0.00	1.56	0.2182
5	74.85	23.59	0.15	1.41	0.1899
10	74.70	23.73	0.30	1.27	0.1629
15	74.57	23.87	0.43	1.13	0.1375
20	74.44	24.00	0.56	1.00	0.1135
25	74.32	24.12	0.68	0.88	0.0909
30	74.20	24.23	0.80	0.77	0.0698
35	74.10	24.34	0.90	0.66	0.0502
40	74.00	24.44	1.00	0.56	0.0320
45	73.91	24.53	1.09	0.47	0.0153
50	73.83	24.61	1.17	0.39	0.0000
Repulsion phase					
0	73.44	25.00	1.56	0.00	−0.0728
5	73.44	25.00	1.56	0.00	−0.0720
10	73.45	24.98	1.55	0.02	−0.0698
15	73.47	24.96	1.53	0.04	−0.0662
20	73.50	24.94	1.50	0.06	−0.0611
25	73.54	24.90	1.46	0.10	−0.0546
30	73.58	24.86	1.42	0.14	−0.0466
35	73.63	24.81	1.37	0.19	−0.0371
40	73.69	24.75	1.31	0.25	−0.0262
45	73.75	24.68	1.25	0.32	−0.0138
50	73.83	24.61	1.17	0.39	0.0000

TABLE 13

Crossing over percentages and values of r when both characters depend upon complementary factors 9:7 and 9:7 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				r
	a''	b''	c''	d''	
	$\frac{18+9p^2}{64}$	$\frac{18-9p^2}{64}$	$\frac{18-9p^2}{64}$	$\frac{10+9p^2}{64}$	$\frac{4p^2-1}{7}$
Coupling phase					
0	42.19	14.06	14.06	29.69	0.4286
5	40.82	15.43	15.43	28.32	0.3729
10	39.52	16.73	16.73	27.02	0.3200
15	38.29	17.96	17.96	25.79	0.2700
20	37.13	19.13	19.13	24.63	0.2229
25	36.04	20.21	20.21	23.54	0.1786
30	35.02	21.23	21.23	22.52	0.1371
35	34.07	22.18	22.18	21.57	0.0986
40	33.19	23.06	23.06	20.69	0.0629
45	32.38	23.87	23.87	19.88	0.0300
50	31.64	24.61	24.61	19.14	0.0000
Repulsion phase					
0	28.13	28.13	28.13	15.63	−0.1429
5	28.16	28.09	28.09	15.66	−0.1414
10	28.27	27.98	27.98	15.77	−0.1371
15	28.44	27.81	27.81	15.94	−0.1300
20	28.69	27.56	27.56	16.19	−0.1200
25	29.00	27.25	27.25	16.50	−0.1071
30	29.39	26.86	26.86	16.89	−0.0914
35	29.85	26.40	26.40	17.35	−0.0729
40	30.37	25.87	25.87	17.87	−0.0514
45	30.97	25.28	25.28	18.47	−0.0271
50	31.64	24.61	24.61	19.14	0.0000

TABLE 14

Crossing over percentages and values of r when one character is determined by complementary factors and the other by duplicate factors—9:7 and 15:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				r
	a''	b''	c''	d''	
	$\frac{33+3p^2}{64}$	$\frac{3-3p^2}{64}$	$\frac{27-3p^2}{64}$	$\frac{1+3p^2}{64}$	$\frac{4p^2-1}{\sqrt{105}}$
Coupling phase					
0	56.25	0.00	37.50	6.25	0.2928
5	55.79	0.45	37.95	5.79	0.2547
10	55.36	0.89	38.39	5.36	0.2186
15	54.95	1.30	38.80	4.95	0.1844
20	54.56	1.69	39.19	4.56	0.1523
25	54.20	2.05	39.55	4.20	0.1220
30	53.86	2.39	39.89	3.86	0.0937
35	53.54	2.71	40.21	3.54	0.0673
40	53.25	3.00	40.50	3.25	0.0429
45	52.98	3.27	40.77	2.98	0.0205
50	52.73	3.52	41.02	2.73	0.0000
Repulsion phase					
0	51.56	4.69	42.19	1.56	−0.0976
5	51.57	4.68	42.18	1.57	−0.0966
10	51.61	4.64	42.14	1.61	−0.0937
15	51.67	4.58	42.08	1.67	−0.0888
20	51.75	4.50	42.00	1.75	−0.0820
25	51.86	4.39	41.89	1.86	−0.0732
30	51.98	4.27	41.77	1.98	−0.0625
35	52.14	4.11	41.61	2.14	−0.0498
40	52.31	3.94	41.44	2.31	−0.0351
45	52.51	3.74	41.24	2.51	−0.0185
50	52.73	3.52	41.02	2.73	0.0000

TABLE 15

Crossing over percentages and values of r when both characters are determined by duplicate factors—15:1 and 15:1 ratios.

PERCENT CROSSING OVER	PHENOTYPIC CLASSES—IN PERCENT				r
	a''	b''	c''	d''	
	$\frac{56+p^2}{64}$	$\frac{4-p^2}{64}$	$\frac{4-p^2}{64}$	$\frac{p^2}{64}$	$\frac{4p^2-1}{15}$
Coupling phase					
0	89.06	4.69	4.69	1.56	0.2000
5	88.91	4.84	4.84	1.41	0.1740
10	88.77	4.98	4.98	1.27	0.1493
15	88.63	5.12	5.12	1.13	0.1260
20	88.50	5.25	5.25	1.00	0.1040
25	88.38	5.37	5.37	0.88	0.0833
30	88.27	5.48	5.48	0.77	0.0640
35	88.16	5.59	5.59	0.66	0.0460
40	88.06	5.69	5.69	0.56	0.0293
45	87.97	5.78	5.78	0.47	0.0140
50	87.89	5.86	5.86	0.39	0.0000
Repulsion phase					
0	87.50	6.25	6.25	0.00	−0.0667
5	87.50	6.25	6.25	0.00	−0.0660
10	87.52	6.23	6.23	0.02	−0.0640
15	87.54	6.21	6.21	0.04	−0.0607
20	87.56	6.19	6.19	0.06	−0.0560
25	87.60	6.15	6.15	0.10	−0.0500
30	87.64	6.11	6.11	0.14	−0.0427
35	87.69	6.06	6.06	0.19	−0.0340
40	87.75	6.00	6.00	0.25	−0.0240
45	87.82	5.93	5.93	0.32	−0.0127
50	87.89	5.86	5.86	0.39	0.0000

TABLE 16

The influence of linkage upon Mendelian ratios.[1]

PERCENT CROSSING OVER	COMPLEMENTARY FACTORS		THREE SUPPLEMENTARY FACTORS		DUPLICATE FACTORS		TRIPLICATE FACTORS	
	$\frac{2+p^2}{4}$	$\frac{2-p^2}{4}$	$\frac{6+3p^2}{16}$	$\frac{10-3p^2}{16}$	$\frac{4-p^2}{4}$	$\frac{p^2}{4}$	$\frac{16-p^2}{16}$	$\frac{p^2}{16}$
Coupling phase								
0	75.00	25.00	56.25	43.75	75.00	25.00	93.75	6.25
5	72.56	27.44	54.42	45.58	77.44	22.56	94.36	5.64
10	70.25	29.75	52.69	47.31	79.75	20.25	94.94	5.06
15	68.06	31.94	51.05	48.95	81.94	18.06	95.48	4.52
20	66.00	34.00	49.50	50.50	84.00	16.00	96.00	4.00
25	64.06	35.94	48.05	51.95	85.94	14.06	96.48	3.52
30	62.26	37.75	46.69	53.31	87.75	12.25	96.94	3.06
35	60.56	39.44	45.42	54.58	89.44	10.56	97.36	2.64
40	59.00	41.00	44.25	55.75	91.00	9.00	97.75	2.25
45	57.56	42.44	43.17	56.83	92.44	7.56	98.11	1.89
50	56.25	43.75	42.19	57.81	93.75	6.25	98.44	1.56
Repulsion phase								
0	50.00	50.00	37.50	62.50	100.00	0.00	100.00	0.00
5	50.06	49.94	37.55	62.45	99.94	0.06	99.98	0.02
10	50.25	49.75	37.69	62.31	99.75	0.25	99.94	0.06
15	50.56	49.44	37.92	62.08	99.44	0.56	99.86	0.14
20	51.00	49.00	38.25	61.75	99.00	1.00	99.75	0.25
25	51.56	48.44	38.67	61.33	98.44	1.56	99.61	0.39
30	52.25	47.75	39.19	60.81	97.75	2.25	99.44	0.56
35	53.06	46.94	39.80	60.20	96.94	3.06	99.23	0.77
40	54.00	46.00	40.50	59.50	96.00	4.00	99.00	1.00
45	55.06	44.94	41.30	58.70	94.94	5.06	98.73	1.27
50	56.25	43.75	42.19	57.81	93.75	6.25	98.44	1.56

[1] When the factorial relationship is known as well as the observed ratio, the percent crossing over can be read directly. The effect of linkage of more than two factors is not considered but this would be possible when three factors are involved in the expression of one character.

PERICARP STUDIES IN MAIZE. III. THE FREQUENCY OF MUTATION IN VARIEGATED MAIZE PERICARP[1]

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INTRODUCTION

Variegated maize pericarp offers good material for studies on mutation as the mutaton rate is very high and significant data can readily be obtained. The studies herein reported were undertaken to determine whether the mutaton rate remained constant through that period in the life history of the plant for which comparative data could be obtained.

MATERIAL AND METHODS

The variegated plants used in these studies were all obtained by outcrossing plants heterozygous for variegated (P^{vv}) and red-cob white (P^{wr}) to white (P^{ww}). Half of the resulting plants were variegated of the factorial constitution $P^{vv}P^{ww}$. The other half were red-cob white and were discarded.

The mutations studied were the mutations from variegated to red. These mutations were tabulated from their somatic expression on the plants themselves. The relative stage in the life history at which the

[1] Paper No. 146, Department of Plant Breeding, CORNELL UNIVERSITY, Ithaca, New York.

mutation took place is indicated by the area affected. Previous work on variegated pericarp (EMERSON 1914, 1917) had shown the visible patches of color on the ear to be due to the same mutational changes which also affected the germ cells. That the changes involved are gene mutations is indicated by the fact that the new forms produced behave as allelomorphs to the parental type and to other members of the same allelomorphic series (ANDERSON 1924).

As described by EMERSON (1917) the mutations observed are of two types:

1. The near-self type in which the color is located in the deeper lying tissue of the pericarp. The glumes of the cob and a patch at the tip of the grain remain white or nearly so. These mutations are heritable and give rise to self-red progeny.

2. The dark-crown type. In this type the color seems to be confined to the epidermis except the tip of the grain which becomes deeply colored. The glumes of the cob, which are made up largely of epidermis, also become red. This type is not inherited presumably because the mutation has taken place in cells which do not give rise to germ cells.

The variegated ears were harvested and examined for mutation areas. Areas covering one grain or more were classified and recorded. Grains with near-self mutation areas covering a considerable fraction of the grain were saved and classified. The remaining grain was shelled and weighed. The number of grains without mutation areas of recorded size was computed from weight and counts of weighed samples.

The plants used in these studies were grown at the Plant Breeding Department of CORNELL UNIVERSITY in 1919 and 1920.

DATA

A tabular presentation of the data on near-self mutation is given in table 1. The original data were tabulated in classes of $\frac{1}{2}$ grain intervals, but the data have been grouped to reduce the number of classes. A similar presentation of the dark-crown data is given in table 2. Dark-crown areas affecting only a fraction of a grain are more difficult to classify so were not recorded.

Many of the larger areas are of indeterminate size because their boundaries do not fall entirely within the areas under observation. They are parts of larger mutation areas including tissue outside of the seed-producing part of the ear. They are the result of mutations taking place earlier than their recorded size would indicate.

TABLE 1

Data on frequency of near-self (heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OF GRAINS IN MUTATION AREA														
		⅛–¼	¼–½	½–1	1	2	3	4	5	6	7	8	9–16	17–32	33–64	65–128
A 1506	37983	968	188	78	39	20		1		1			1			
A 1507	6009	99	42	16	10	1										
A 1508	11185	228	82	39	17	4	1									
A 1510	32383	834*	229	76	39	15				1						
A 1512	7299	264	25	21	5	3		1								
A 1513	8028	193	20	15	10	3										
A 1514	13993	389	70	28	7	2	1				1					
A 1515	14922	378	124	44	20	5	3									
A 1516	25001	447	112	72	23	2	3	1								
A 1517	25204	1041	140	48	17	13	1									
A 1518	25706	700	111	51	33	10										
A 1519	29790	884	148	86	34	8	3		1							
A 1520	37265	774	173	56	36	8	1	2					2			
A 1740	33159	412	214	105	31	14			1			1	1			
A 1742	1792	15	12	2	1											
A 1746	29768	502	265	98	37	11	1	1		1						
A 1747	15089	214	107	35	17	5	1						1		1	
A 1757	8686	94	32	13	9	1										
A 1762	1067	11	6	1	1	1										
A 1763	4012	55	36	14	6	1										
A 1765	13173	201	70	38	1	7							1			
A 1770	41325	368	133	14	46	7	3						2	1	1	
Ey 843	37580	2008	247	145	68	9	4	1				1	3	1		
Ey 844	782	29	5	0	1											
Ey 847	50102	808	183	64	20	4	3					1				
Ey 848	7631	136	25	11	7	2			1							
Ey 851	48147	1509	386	164	88	23	2	3	3	1		1	3			
Ey 852	3754	115	23	12	6	2	2									
Ey 855	4124	163	25	16	7											
Ey 856	39757	740	195	59	45	6	1				1		1			
Ey 860	901	32	8	3	2											
Ey 862	2052	86	19	7	4	1										
Ey 864	2123	77	19	10	5	1							1			
Ey 868	1191	41	18	2	9	1										
Ey 871	1062	34	7	1	1	1										
Ey 874	33233	1281	299	122	72	20	4	1	1		1			1		
Ey 875	1580	38	11	4	1											
Ey 876	543	26	2	1	0											
Ey 877	2296	67	10	7	8	4		1								
Ey 878	29541	1428	181	67	50	13	6	2								
Ey 883	654	19	3	2	0											
Ey 886	4513	160	32	10	10	5	2									1
Ey 890	1277	33	8	4	3											
Total	695682	17921	4045	1661	846	233	42	14	7	4	3	4	16	3	2	1

* Estimated. Observed number not recorded.

TABLE 2

Data on frequency of dark-crown (non-heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OF GRAINS IN MUTATION AREA																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-32	33-64	65-128	129-256	257-512
A 1506	37983	748	42	12	10	4	4			2	1				1		1	3	2			
A 1507	6009	130	2		1		2															
A 1508	11185	278	20	7	3			1												1		
A 1510	32383	808	41	14	6	6		2		1					2			1				
A 1512	7299	93	10	1							1											
A 1513	8028	68	6	1																		
A 1514	13993	139	12	1	1	1	1	1														
A 1515	14922	187	4	2		1	1	1										2				1
A 1516	25001	377	11	4	3	1	1	1	3	1					1			1			1	1
A 1517	25204	447	23	7		1	1			1			1	1		1			1			
A 1518	25706	525	29	4	1	2	1	1		1	1			1	1	1				1		2
A 1519	29790	498	31	6	2					2				1	1				1	1		
A 1520	37265	588	25	10	4	4	3	1			1		1	1				3				
A 1740	33159	484	32	11	4	3	1		1	1					1		1	1				
A 1742	1792	6	1		1			1			1							2				
A 1746	29768	318	13	4	3	2	3			1								1				
A 1747	15089	188	14	3	1				1													2
A 1757	8686	45	4	1	1														2			1
A 1762	1067	8	1																			
A 1763	4012	38	1				1															
A 1765	13173	152	6	6		1	1															
A 1770	41325	526	31	6	3	2	1		1	1		1						2				

TABLE 2 (continued)

Data on frequency of dark-crown (non-heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OE GRAINS IN MUTATION AREA																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-32	33-64	65-128	129-256	257-512
Ey 843	37580	291	35	41	7	2	6	1	1	1			1	1	1		1	3	1			
Ey 844	782	2																				
Ey 847	50102	214	13	3	2	1	2								2	1						
Ey 848	7631	35	3	2	1								1									
Ey 851	48147	783	65	26	17	6	6	2		1	3	3				1		1	2		2	
Ey 852	3754	59	6	4		1	1															
Ey 855	4124	94	6		3	1										1						
Ey 856	39757	334	51	9	5	4	2	1			1	1	1	1				2	1			
Ey 860	901	4	1																			
Ey 862	2052	38	7	3																		
Ey 864	2123	31	4										1									
Ey 868	1191	7	1			1																
Ey 871	1062	13	1																			
Ey 874	33233	658	36	15	6	2	3	2	2	1	1	2			1			4	2			
Ey 875	1580	26																				
Ey 876	543	6																				
Ey 877	2296	28	4	3			1															
Ey 878	29541	313	35	10	7	3	1	3		1	1	1	3			1		1		1		
Ey 883	654	3	4	1																		
Ey 886	4513	54	7	2		2	1	1		2												
Ey 890	1277	21	2										1									
Total	695682	9665	640	192	92	51	44	19	9	17	11	8	10	6	11	6	3	27	12	4	3	7

COMPARISON OF NEAR-SELF AND DARK-CROWN FREQUENCIES

Table 3 gives a comparison of the frequency of occurrence of equivalent sized near-self and dark-crown mutation areas. The frequencies from tables 1 and 2 are grouped into classes such that the limits of one class are twice those of the preceding. This is done to avoid the extremely low frequencies of the individual classes of large area.

TABLE 3

Comparison of frequencies of near-self (heritable) and dark-crown (non-heritable) mutations.

MUTATION AREA	NEAR-SELF	DARK-CROWN
0.5– 1	2509	9665
1 – 2	233	640
3 – 4	56	284
5 – 8	18	123
9 – 16	16	72
17 – 32	3	27
33 – 64	2	12
64 –128	1	4
129 –256	0	3
257 –512	0	7
Total	2838	10837

The frequencies for the dark-crown series average about 3.8 times as great as those for the corresponding near-self classes. This may mean that the mutation rate is actually this much greater in those meristem cells which give rise to the tissues affected by the dark-crown mutations. But since the method of growth of the tissues concerned may not be comparable, this cannot be considered proved.

THE THEORETICAL FREQUENCY RELATION BETWEEN DIFFERENT CLASSES FOR A CONSTANT MUTATION RATE

If the mutation rate were constant the number of mutation areas of small size would be more frequent than those of large because there would have been more cells present which might have mutated. Also the numerical class interval chosen is not one that gives an equivalent length of time for mutations to take place. The exact numerical relation cannot be calculated due to the irregularity of growth. But a calculation based on regular multiplication of cells will approximate the true relationship sufficiently closely for comparison with the observed data. A mutation

affecting twice the area of another may be assumed to have occurred one cell generation earlier when the number of cells was only one-half as great. The time interval, expressed in cell generations, may be calculated on the same basis. An error, probably very small, is introduced in the near-self series by growth in the third dimension during the period under observation.

On the above basis, the relative number of available cells at the time of mutation varies inversely as the size of the mutation area, and the time interval varies as the differences between the logarithms of the numerical sizes of the mutation areas.

CALCULATION OF COMPARATIVE MUTATION RATE

The ratios of the observed frequencies to the calculated relative frequencies give us a measure of the relative mutation rate for the period of several cell generations during which the recorded mutations took place. The calculation of this comparative mutation rate may be simplified by a process which is equivalent to making the theoretical equal to unity and multiplying the observed frequencies by the appropriate factor. The simplified calculation is made as follows: (a) The observed frequencies are multiplied by their respective class values. (b) These products are then summed into classes having equal logarithmic intervals. The interval chosen is such that the numerical limits of one class are twice those of the preceding. This interval is such that each class then represents a cell generation. This summation greatly condenses the long series of low frequency classes.

The number of units of area available for mutation is slightly altered by the fact that once an area has mutated it is no longer available for mutation. This error can be corrected by dividing each product by the difference between the total number of grains and the number included in the larger mutation areas. The change due to this correction is not sufficient to alter the calculated rates more than a few percent but for the sake of completeness both the uncorrected and corrected figures are given in the tables and graphs which follow.

COMPARATIVE RATE OF MUTATION

The comparative rate of mutation calculated for the near-self series is given in table 4. The rate of mutation is expressed as the number of cells mutating per hundred thousand during a period equivalent to one cell generation. A graph of the mutation rate for the near-self series is given

TABLE 4

Mutation rate of near-self (heritable) mutations expressed as the number of mutations per hundred thousand cells during one cell generation.

MUTATION AREA	RATE	CORRECTED RATE
0.12– 0.25	644.0	668.7
0.25– 0.5	290.7	300.0
0.5 – 1.0	360.4	365.4
1.0 – 2.0	67.0	67.7
2.0 – 4.0	26.2	26.4
4.0 – 8.0	16.1	16.2
8.0 –16.0	26.6	26.8
16.0 –32.0	9.5	9.5
32.0 –64.0	11.4	11.4

in figure 1. The graph is arranged so that successive cell generations read from left to right. The last class is of little value because of difficulty of classifying. The irregularity in the curve near the right end is largely due to the fact that the class limits adopted do not conform to the original

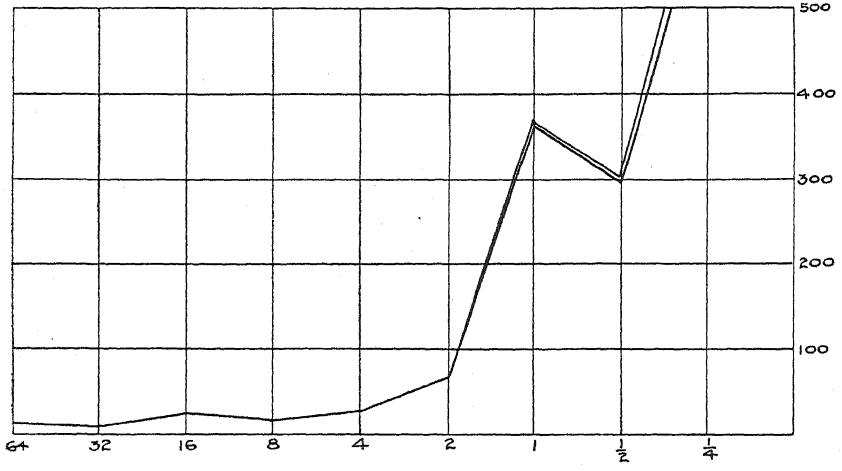


FIGURE 1.—Graph of near-self mutation rate from table 4. Size of mutation areas plotted as abscissae, mutation rates as ordinates.

classification. Table 5 and figure 2 give the corresponding data for the dark-crown series. The curve is smoother because there is no discrepancy in the summation of classes. Mutation areas affecting more than 64 grains for the near-self and more than 128 grains for the dark-crown series are omitted as most of these extended beyond the limits of the ear on which they occurred and were consequently of indeterminate size.

TABLE 5

Mutation rate of dark-crown (non-heritable) mutations expressed as the number of mutations per hundred thousand cells during one cell generation.

MUTATION AREA	RATE	CORRECTED RATE
1	1389.3	1408.7
1 - 2	184.0	190.4
2 - 4	135.7	137.1
4 - 8	104.1	105.0
8 - 16	120.9	121.8
16 - 32	93.1	93.7
32 - 64	82.7	83.1
64-128	58.5	58.8

The rate of mutation for both series shows a great increase within the period covered by these observations. The increase is probably a gradual one, as the sharp changes in the graphs are at least largely due to difficulties encountered in the classification of mutation areas of small size. The

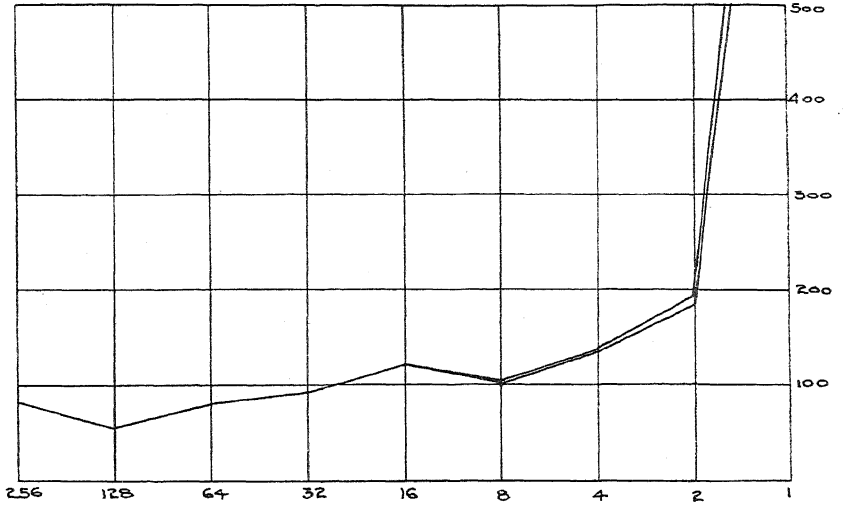


FIGURE 2.—Graph of dark-crown mutation rate from table 5. Size of mutation areas plotted as abscissae, mutation rates as ordinates.

values recorded for the larger areas are a little too large due to the inclusion of mutation areas which were not completely included in the seed-bearing part of the ear on which they occurred.

As the recurrent mutations involved in variegation are perhaps not comparable to other gene mutations, it cannot be inferred from these data

that other gene mutations will show a similar increasing frequency during ontogeny. In this connection, it is well to remember that in the conspicuous example of recurrent change in the bar gene of *Drosophila melanogaster*, STURTEVANT has shown that the changes are due to unequal crossing over in the immediate vicinity of the bar gene (STURTEVANT 1925). This is in marked contrast to the mutations recorded in this paper which occurred in somatic tissue long before the maturation divisions.

SUMMARY

Somatic mutations to red in variegated maize pericarp were classified as to the area affected. The near-self (heritable) and dark-crown (non-heritable) series of mutations were recorded separately.

The dark-crown mutations were about 3.8 times as frequent as the near-self mutations affecting equal areas.

Both the near-self and dark-crown series show an increasing rate of mutation during the period of ontogeny covered by these studies.

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ALCOHOL AND BODY WEIGHT IN THE ALBINO RAT

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This paper deals with one phase, effect upon body weight, of an experiment designed to test the effects of alcohol, both germinal and somatic, upon the albino rat. The data given cover ten successive generations of alcoholic administration. An introductory paper by HANSON and HANDY (1924) gives in detail the methods employed.

Suffice it here to say that: the fume tank method devised by STOCKARD was used in administering the alcohol; treatment was begun in each generation when the animals were twenty days of age and continued until they were one hundred days old, except in the first generation where the treatment continued for a period of one year; the treatment might be described as severe, lasting each day until the animals were unable to stand upon their feet; the general environmental conditions for test and control rats were identical—the alcoholic treatment being the single differential between the two groups; both treated and control animals were the descendants of a single pair of WISTAR INSTITUTE semi-inbred rats (Tyler strain); all matings throughout the experiment were sister-by-brother within the litter; the animals were weighed to a tenth of a gram at birth, at twenty days of age and every ten days thereafter until the last weighing at age one hundred days; all weighings were made by the senior author thereby reducing the personal equation to a minimum.

After the first generation which was small in numbers an attempt was made to base each body weight constant upon at least fifty rats. In practice, however, this was not always possible, the number falling below in some cases and rising considerably above in others. It is believed that in the rigid character of the controls, the number of treated generations, the strictly inbred character of the blood-lines, and the total number of animals (1825 at twenty days of age) involved, this experiment may contribute something to the alcohol problem.

Table 1 gives the data for the totals of the ten generations of control and test animals. The means are based on large numbers of rats, and these numbers are given to the left of each mean in the table. The data for the males and females are given separately.

Males: In males at twenty days of age the control mean is only 0.27 of a gram greater than the corresponding mean in the treated. As this

difference is less than its probable error it seems clear that alcohol fumes, administered to rats over a period of five years and including ten generations, has not impaired the ability of these animals to produce young of equal weight with those of the controls. It will be recalled that treatment

TABLE 1

A table showing body weight constants for the total ten generations at various ages.

AGE IN DAYS	NUMBER RATS	CONTROL	NUMBER RATS	TREATED	DIFF. / P. E
		Males			
20	408	25.17±0.22	490	24.90±0.21	——
40	392	64.39±0.58	434	58.57±0.59	7.09
60	364	113.71±1.06	374	102.76±1.01	7.52
80	317	156.70±1.40	317	145.55±1.44	4.82
100	248	190.85±1.70	227	177.31±1.86	5.42
		Females			
20	454	24.14±0.22	473	25.07±0.21	3.10
40	412	59.66±0.52	421	55.89±0.50	5.24
60	402	99.85±0.86	386	91.01±0.85	7.31
80	374	130.11±1.00	324	126.30±1.08	2.59
100	266	150.79±1.25	170	144.18±1.68	3.19

began at twenty days of age, so that comparisons between treated and controls at this age are based upon rats which have not themselves been treated. Any inherited growth deficiency due to alcoholization of parents and grandparents should be evident at this age. HANSON and HEYS also have shown elsewhere that in litter size and birth weight the offspring of the treated are fully equal to those of the controls.

After the weighing at twenty days those rats in the alcoholic line of descent received their first treatment in the fume tanks and both treatment and weighings were continued until one hundred days of age was attained. The comparison between the two means at every subsequent weighing shows a large significant difference, reaching its maximum at sixty days where the difference is 7.52 times its probable error. This difference at every age above twenty days is in favor of the controls. The conclusion that alcoholic treatment has adversely affected the growth rate seems valid—in other words, descendants with an alcoholic ancestry weigh just as much at birth and at twenty days as the corresponding controls, but these same descendants show a marked falling off in body weight soon after they themselves become the subjects of treatment.

However, as above indicated, these same alcoholic-stunted animals for ten successive generations do not lose their inherent capacity to produce young which at the age of twenty days have normal body weight. Therefore, the effects of alcohol on body weight as shown by these data do not go beyond the soma.

Females: At the ages of forty to one hundred days inclusive the results with females are identical with those of the males and the discussion above applies equally here.

At twenty days the situation is different in this respect: that the young of treated ancestry actually have a larger mean weight by 0.93 of a gram than the controls. At all later ages, due to the direct action of the alcohol, the means of the treated are significantly lower than those of the controls.

Not only does an alcoholic parentage not inhibit growth up to twenty days of age, but actually in the case of females, due to selective elimination of inferior germ cells, differential prenatal mortality or some other unknown cause, produces twenty-day offspring with a significantly greater mean body weight.

It is worthy of notice that at twenty days in both males and females the mean body weight of treated and controls shows a difference of less than a gram although the two groups have been carried in separate lines of descent for a period of over five years.

MACDOWELL treated white rats with alcohol primarily with a view to testing their learning reactions in a Watson maze. Data on growth and fertility were taken at the same time and these have been published separately. The paper on growth (1922) discusses the effect of alcohol on body weight in several groupings of his animals variously described as "Treated rats," "Untreated rats from treated parents," "Untreated rats from untreated parents and treated grandparents," and "Treated rats from treated parents."

His first and last groups are most comparable to our experiment and they alone will be discussed. In the group termed by MACDOWELL, "Treated rats," his conclusion is that the treatment of white rats with maximum doses of alcohol tends to retard growth as compared with their untreated brothers and sisters. His data are in every particular, as regards this one group, identical with ours. In his group known as "Treated rats from treated parents," he found no significant differences between treated and controls. These latter data are based on thirty-one treated rats and thirty-four controls. Our own experiment might be described as treated rats from treated parents and grandparents for ten generations and in-

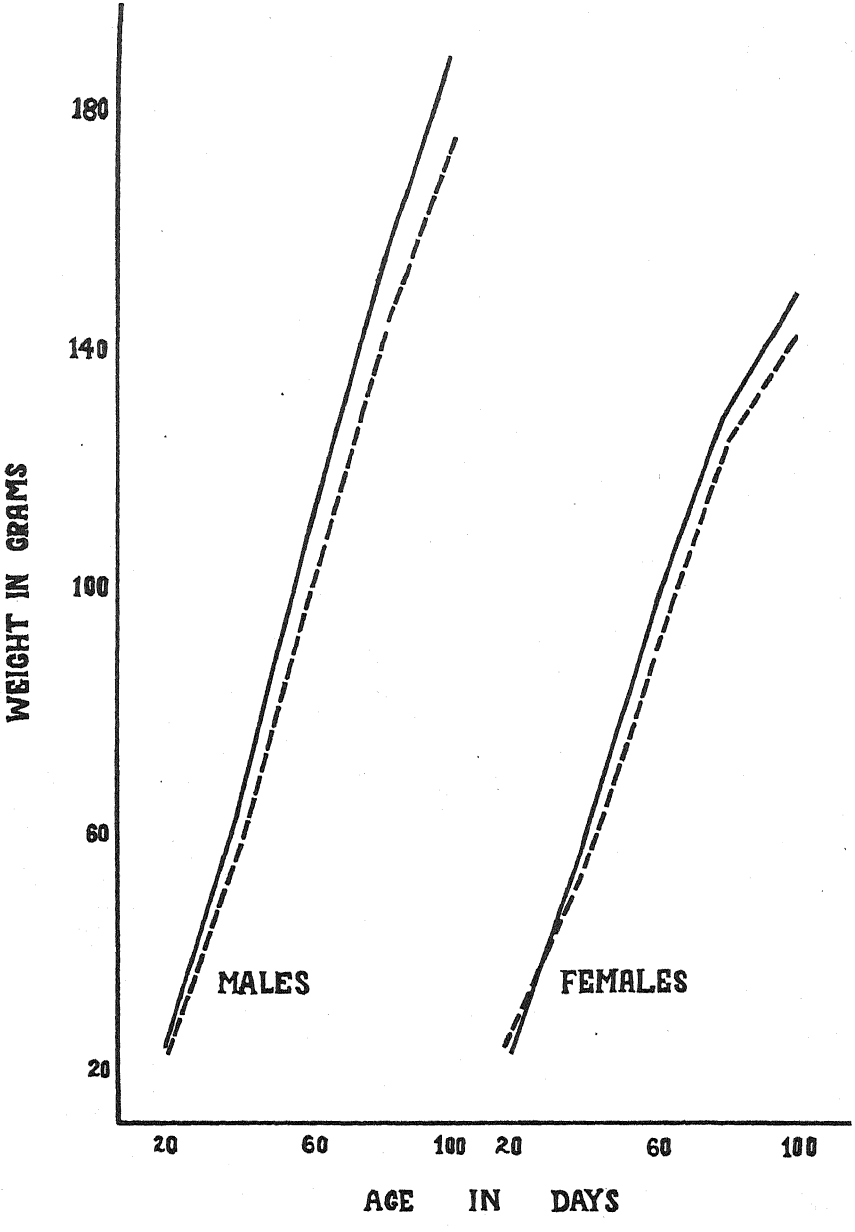

180
140
100
60
20
WEIGHT IN GRAMS
MALES
FEMALES
20
60
100
20
60
100
AGE IN DAYS

FIGURE.—1

volves nearly two thousand rats. That our results in this particular instance are not in agreement with MACDOWELL'S may well be due to the difference in the magnitude of the two experiments.

ARLITT (1919) claimed that alcohol retarded body growth and that "the defective body weight acquired by alcoholized rats is also inherited." MACDOWELL has ably criticised ARLITT'S methods and apparently undermined her conclusions. Our data, based on the same species, are, of course, further refutation if any be needed.

STOCKARD and PAPANICOLOAU (1917) found that in guinea pigs the normal offspring weigh more and for a time grow more rapidly than the young of alcoholic pigs.

PEARL (1917) describes the effects of both ethyl and methyl alcohol on chickens. After fifteen months of treatment the alcoholic birds were 9.9 percent heavier than untreated control birds of the same average age, and this increase in weight of the treated birds is apparently due entirely to deposition of body fat and not a fatty infiltration of any of the visceral organs.

CONCLUSION

1. Alcohol has a retarding effect upon the growth rate of albino rats.
2. This effect is not in any degree transmitted to their offspring even after ten successive generations of exposure to the fumes.

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PARTHENOGENESIS AND THE INHERITANCE OF COLOR PATTERNS IN THE GROUSE LOCUST, *TELMATETTIX AZTECUS* SAUSSURE[1]

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MATERIAL AND METHODS

Specimens of grouse locusts, identified by Doctor A. N. CAUDELL as *Telmatettix aztecus* Saussure, were secured along Shoal Creek, Austin, Texas, in August, 1922. By breeding analyses it was determined that there were four elementary patterns as follows: (1) A mottled gray closely resembling the +/+ of *Apotettix eurycephalus* (NABOURS 1925), and the ensemble of indistinguishable factors responsible for it, are represented by the symbol +/+; (2) a dense black extending over the anterior pronotum and on the femora of the jumping legs is indicated by the letters *Bl;* (3) a grayish white over the anterior pronotum and lateral lobes, resembling *C*, of *Paratettix texanus* (NABOURS 1917), although the posterior pronotum and legs are quite yellow, is represented by the letter *C*; (4) a dark greenish gray over the pronotum, including vertex, eyes and femora, with lighter lateral lobes, ranging almost to white, is designated by the letter *H*. In October, 1923, new specimens were secured from the same region, along Shoal Creek, and these included a new pattern; (5) a dull brick red covering the vertex, eyes, pronotum and posterior femora. This is designated by the letter *R*.

In January, 1923, Mr. DAVIS sent some specimens of this species from Pasadena, California. Only two individuals, females +/+ and +/*C*, respectively, lived to be mated. They both produced offspring from males of the Texas stock. None of the progeny from the +/+, Pasadena female, was bred further. Three individuals from the +/*C* female (Pasadena) were bred and their progenies were widely used in later matings. It is to be noted, however, that none of the females producing parthenogenetic offspring, described farther on, had an ancestry which traced back to either of these Pasadena females.

All adult individuals of this species (*Telmatettix aztecus*) have had long wings and pronota, as against *P. texanus* and *A. eurycephalus*, individuals of which frequently have had short wings and pronota (NABOURS 1914, 1917, 1925).

[1] Contribution No. 96 from the Department of Zoology, Agricultural Experiment Station, KANSAS STATE AGRICULTURAL COLLEGE.

They were bred casually and in small numbers during the year 1922-23, rather intensely and in considerable numbers in 1923–24, and only in small numbers since (till 1927).

They are bred in the greenhouse, and the mating jars used are the regular 8″×12″ glass cylinders set in pots of earth and covered with screen wire lids. The food, consisting mainly of the filamentous algae, is placed on small earthern pots partly buried upside down in the soil (NABOURS 1914, 1925).

The length of time required for breeding a generation varies with the time of the year and conditions of environment, the best season being the spring and early summer months. On the average, four succeeding generations a year may be bred in the greenhouse at Manhattan, Kansas. On hatching, the young are transferred, about 25 each, to larger similar cages. When of sufficient size for the color patterns to be easily distinguished, preferably during the third instar, they are recorded. As they become adult, the desired matings are made, and those remaining are again recorded, and preserved in 95 percent alcohol, in glass tubes sealed with a torch.

The experimental breeding of this species is much more difficult than is that of *P. texanus* and still more arduous than of *A. eurycephalus*. A large proportion of the matings are not productive, due usually to the early death of one or both individuals. During the whole period 1922–27, only 116 of the 187 matings made were productive. During the one year of most intensive breeding, May, 1923, to June, 1924, only 53.9 percent of the matings were productive; the range throughout the months of that year is indicated in table 1.

TABLE 1

Matings made compared with number productive—year 1923–24.

MONTH	MATINGS MADE	PRODUCTIVE MATINGS
June	4	2
July	2	1
August	5	3
September	18	7
October	28	13
November	5	1
December	2	1
January	1	1
February	9	3
March	8	3
April	21	17
May	12	10
Totals	115	62
53.9 percent productive		

The number of individuals recorded fell far short of the number of young hatched and transferred from the mating jars. During the whole period of five years, only 50.6 percent or 2850 of the 5634 offspring transferred, were finally recorded. However, during the year June, 1923, to July, 1924, when better attention was given them, 55.19 percent of those transferred had their color patterns recorded. Table 2 shows the distribution of the mortality throughout that year.

TABLE 2

Number transferred compared with number recorded—year 1923-24.

MONTH	NUMBERS TRANSFERRED	NUMBERS RECORDED
July	214	110
August	64	30
September	0	0
October	154	61
November	317	96
December	28	10
January	50	19
February	313	173
March	155	94
April	264	169
May	504	272
June	1,448	904
Totals	3,511	1,938
55.19 percent recorded		

SEGREGATION OF FACTORS IN THE MATED INDIVIDUALS

(Males on left, females on right of hyphen)

The segregation of the factors of all the individuals heterozygous for the color patterns is shown in table 3. Referring to the table; beginning at the left with the item *Bl/C*, for example, all males bred gave 166 carrying *Bl*:165 carrying *C*; the females gave 192 *Bl*:174 *C*; both males and females gave 358 *Bl*:339 *C*, in a total of 697 gametes.

In this paper, as in a previous one (NABOURS 1925), the symbol + (or +/+) is made to serve, as it were, a double role. In tables 3 and 4, and wherever so used, + opposite a symbol for a dominant factor (for example +/*C*), refers simply to the normal recessive allelomorph of that factor. In case none of the dominant factors is present, +/+ refers to the ensemble of indistinguishable factors that produces the mottled gray pattern, common to all species of the Tettigidae so far used in the experiments, and which has been found generally recessive to the more striking patterns. This mottled gray pattern, +/+, is much more

abundant among all the species observed in nature than are the more prominent dominant patterns. Therefore, it might be very well called the "wild type," though all the dominant patterns are also found in nature.

TABLE 3

A summary of the segregation of factors of heterozygous males and females of Telmatettix aztecus.

HETEROZYGOTES	SEGREGATION OF FACTORS IN MALES		SEGREGATION OF FACTORS IN FEMALES		SEGREGATION OF FACTORS IN MALES AND FEMALES COMBINED		TOTAL GAMETES
+/*C*	+	*C*	+	*C*	+	*C*	
	153	157	213	228	366	385	751
+/*Bl*	+	*Bl*	+	*Bl*	+	*Bl*	
	312	276	96	96	408	372	780
+/*H*	+	*H*	+	*H*	+	*H*	
	67	53	67	60	134	113	247
+/*R*	+	*R*	+	*R*	+	*R*	
	131	121	145	167	276	288	564
Bl/*C*	*Bl*	*C*	*Bl*	*C*	*Bl*	*C*	
	166	165	192	174	358	339	697
Bl/*H*	*Bl*	*H*	*Bl*	*H*	*Bl*	*H*	
	0	1	32	24	32	25	57
Bl/*R*	*Bl*	*R*	*Bl*	*R*	*Bl*	*R*	
	0	0	99	124	99	124	223
C/*H*	*C*	*H*	*C*	*H*	*C*	*H*	
	211	215	96	68	307	283	590
C/*R*	*C*	*R*	*C*	*R*	*C*	*R*	
	149	130	58	62	207	182	389
H/*R*	*H*	*R*	*H*	*R*	*H*	*R*	
	33	39	24	26	57	65	122
Totals	1222	1147	1022	1029	2244	2176	4420

In addition to the data summarized in the table above, there was one mating of a male +/*C* to a female +/*C*. This gave 44 +/*C* and *C*/*C*: 14 +/+ individuals, a close approximation of the 3:1 ratio.

PARTHENOGENESIS

Parthenogenesis has been found to occur in *Apotettix eurycephalus* (NABOURS 1919, 1925) and *Paratettix texanus* (NABOURS and FOSTER, abstract, 1925).

Virgin females of *T. aztecus* were kept by themselves from time to time to test them for parthenogenesis (NABOURS and SNYDER 1924). Two or three females of a kind were placed together in each cage, except once when one female was alone.

Of 15 groups of females thus separated from males, 12 gave from 1 to 112 offspring. The first four items of table 4 are hardly worthy of a place in the table, because early death prevented complete records, but they were undoubtedly cases of parthenogenesis. In another cage, in which two females had been kept, unhatched eggs were found. The other eight items gave quite definite results. The two $+/C$ females, (9), table 4, that gave 49 females, $+/+$, and 53 females, C/C, were among the highest producers parthenogenetically (112 offspring transferred) that have been recorded among the individuals of any of the three species.

The one C/C female produced parthenogenetically, item (6), in table 4, was mated to an H/R male, and gave C/H 5–7:C/R 3–7. Another C/C female from a parthenogenetic progeny, item (9), table 4, was mated to a $+/Bl$ male, and gave $+/C$ 0–4:Bl/C 2–2.

TABLE 4

Parthenogenesis in Telmatettix aztecus

(Males on the left; females on the right; figure on right of second hyphen indicates sex not recorded).

HETEROZYGOUS FEMALES	PARTHENOGENETIC OFFSPRING
(1) 2 *Bl/C*	*Bl/Bl* 0–0–1
(2) 2 *Bl/C*	*Bl/Bl* and *C/C* 0–3–12
(3) 2 *+/Bl*	+/+ 0–0–1
(4) 2 *+/C*	*C/C* 0–0–2
(5) 2 *Bl/C*	*Bl/Bl* 0–1
(6) 2 *+/C*	*C/C* 0–1
(7) 2 *Bl/C*	*C/C* 0–1
(8) 1 *+/Bl*	+/+ 0–8, *Bl/Bl* 0–8
(9) 2 *+/C*	+/+ 0–49, *C/C* 0–53
(10) 3 *C/H*	*C/C* 0–7, *H/H* 0–5
(11) 3 *+/R*	+/+ 0–1, *R/R* 0–1
(12) 3 *+/C*	+/+ 0–3, *C/C* 0–3

DISCUSSION OF PARTHENOGENESIS

PEACOCK and HARRISON (1926) have analyzed the data of the experiments showing parthenogenesis in *Apotettix eurycephalus* (NABOURS 1919, 1925) and found from the records that all the unmated individuals that gave offspring could be traced back to hybrids from specimens of a Mexican variety crossed with individuals of a variety from Texas. They

concluded, apparently with good reasoning, that this evidence supported their previously stated hypothesis (PEACOCK and HARRISON 1925) that parthenogenesis was consequent upon hybridity. But in *Telmatettix aztecus*, herein reported, *the individuals that gave parthenogenetic progeny were all descended directly and exclusively from specimens captured on a very small area along Shoal Creek, Austin, Texas.* None of the progeny from the Pasadena, California, specimens was mixed with those that gave offspring parthenogenetically.

However, the fact that parthenogenesis was, in these cases, confined to stocks that had come from one small area does not eliminate the possibility of hybridization, even there. This is a rather widely distributed species, and the chances for individuals of varieties to be carried by the wind, or other agencies, from place to place should not be overlooked.

An extraordinary range in markedly differing elementary color patterns, and a vast array of possibilities for combinations into pattern complexes (see plates, NABOURS 1917, 1923, 1925) are exhibited by practically all the species of the Tettigidae. They undoubtedly also have other equally, or more potent characteristics, even though less readily discernible, in just as great, or greater variety and hybrid complexity. Therefore, the results of the parthenogenetic breeding of *T. aztecus*, reported here, can hardly be made to bear on the hypothesis advanced by PEACOCK and HARRISON, certainly not in the direction of refuting it. In fact, it does not appear now just how this interesting hypothesis might be put to a critically experimental test with any of the species of the grouse locusts, from whatever small area breeding stock might be collected, for they are all probably complexly hybridized.

CYTOLOGY

In *Paratettix texanus*, *Apotettix eurycephalus*, *Acrydium granulatum*, *Tettigidea parvipennis* and other grouse locusts (Tettigidae), there have been found uniformly thirteen spermatogonial chromosomes (HARMAN 1915, ROBERTSON 1916). Cytological preparations for the study of spermatogenesis in *T. aztecus*, prepared with the aid of Doctor MARY T. HARMAN, exhibit six and seven chromosomes in the nuclei of the secondary spermatocyte divisions.

SUMMARY

1. A series of four factors for dominant color patterns and their normal recessive have been analyzed. It appears that these factors are confined to the one series, or one pair of chromosomes, and that they are alterna-

tive, or so closely linked that no crossing over has occurred during the experiments.

2. Gynogenetic parthenogenesis occurs, in which segregation of the factors of heterozygotes takes place as in bisexual reproduction.

3. Females descended directly from individuals collected on a very small area gave offspring parthenogenetically. The results of this experiment, for the reasons given, are, therefore, neutral with respect to the hypothesis of PEACOCK and HARRISON that parthenogenesis is consequent upon hybridity.

4. The chromosome numbers have been found to be six and seven in the secondary spermatocyte division cells.

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LINKAGE STUDIES IN RICE[1] *

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INTRODUCTION

According to the chromosome theory, all genes on the same chromosome are linked, unless crossing over takes place. If this postulate is true, we should expect to find no more linkage groups than there are haploid chromosomes in the variety or species in question. Thus far, *Drosophila melanogaster* is the only species in the animal kingdom in which all known genes have been found to fall into one or another of the four linkage groups, each probably corresponding to one of the four haploid chromosomes. In the plant kingdom, *Zea mays* is the only species in which the linkage relations of Mendelian characters have been studied extensively.

In rice, the common species, *Oryza sativa L.*, has twenty-four chromosomes (KUWADA 1910, NAKATOMI 1923). We should expect, therefore, to find twelve linkage groups in the common races of rice. Investigations in this respect, however, have just begun. PARNELL (1917) observed the

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association between purple lining of the internode and purple glumes, and also between purple stigma and purple axil, whereas green internode and glumes are associated with purple stigma and axil. He further observed (1922) that purple pericarp color belongs to the same pattern. No satisfactory data, however, have been published regarding the chromosomal relations of the genes responsible for the characters just mentioned. NAGAI (1921) observed close association between the purple awn and the reddish-brown testa. HECTOR (1922) showed that the color of the pericarp is due either to the same factor which is responsible for ligule color or to a factor completely linked with it, and that the fact that a few plants were found with colored ligules and white grains is evidence in favor of the latter view. HECTOR (1922) further found that certain color characters on the vegetative parts of the plant are grouped in patterns or systems which are inherited together, segregating as if they were due to a single factor or due to the same interacting factors. In four cases, however, he observed that some patterns were altered. (1) In the cross Noachur × Pookhi, the pattern "colored internode and stigma" was altered three times out of 1,199 plants examined, giving three plants in F_2 with "colored stigma and green internode." (2) In the same cross, the pattern "colored leaf-sheath and apiculus" changed twice out of 1,199 plants giving two plants in F_2 with "colored apiculus and green leaf-sheath." (3) In the cross Bailabkri × Pookhi the pattern "colored leaf-sheath, internode and stigma" was altered twenty-four times out of 4,687 plants examined, giving twenty-four plants with "colored leaf-sheath and stigma but green internode." (4) In the cross Agartollah × C_{25}, the pattern "colored leaf-sheath, pulvinus, auricles, internode, glumes, apiculus" changed once out of 4,669 plants examined, namely, one plant was found with color in the glumes and apiculus, but with green leaf-sheath, internode, pulvinus and auricles. In all the cases thus far reviewed, while there is some indication of association between certain characters, no linkage group is definitely established.

In three instances, however, the linkage relation seems to be clear. The first of these is between the factor for awn color and the glutinous gene. In the cross, Tamanishiki × Shinriki, TAKAHASHI (1923) found that the dominant factor for awn color (*R*) is coupled with the non-glutinous factor (*U*), giving about twenty-one percent crossing over. The exact percentage of crossing over, however, is not certain, for NAGAI (1926) found 21.7 percent crossing over in one cross and 14.3 percent crossing over in another cross. Of course, the latter case may be concerned with a different factor. The second instance of linkage is given by YAMAGUCHI

(1926) who found that the factor for apiculus color (*S*) is coupled with the non-glutinous gene giving about 20–22 percent crossing over. Just recently, YAMAGUCHI (1927) found that the factor (*F*) for flowering time is also linked with the glutinous gene. The exact locus, however, is not certain.

The present studies were started in the winter of 1924. Some twenty-five factors were studied and their chromosomal relations determined so far as practicable. Since it is not practical to make backcrosses with this plant, the linkage relations were analyzed exclusively from data obtained from the F_2 generation. The varieties used have been briefly described in an earlier paper by the author (CHAO), but a few important characters are pointed out in the following list.

Variety	*Characters*
100	Glutinous endosperm; awnless; colorless apiculus and glumes.
200	Non-glutinous endosperm; long awn; tawny apiculus and glumes.
300	Glutinous; colorless apiculus, stigma and leaf-sheath.
400	Non-glutinous; colored apiculus, stigma and leaf-sheath.
600	Non-glutinous; colorless apiculus, stigma and leaf-sheath; red brown pericarp; colorless ligule, auricle, and internode; light hull with brown furrows.
800b	Glutinous; colored apiculus, stigma and leaf-sheath; purple pericarp; purple ligule, auricle and internode; light hull without brown furrows.
4269	Glutinous; long spikelet and long glumes; colorless apiculus, stigma and leaf-sheath; colorless ligule, auricle and pulvinus; purple pericarp.
4957	Non-glutinous; short spikelet and short glumes; red apiculus; purple stigma; leaf-sheath purple lined; colorless ligule, auricle and pulvinus; white pericarp.

EXPERIMENTS AND RESULTS

Relation between the awn and the glutinous character

Concerning the inheritance of the awned and awnless character in rice, YAMAGUCHI (1926) reported a simple Mendelian 3:1 ratio. NAGAI (1926) reported three cases, one segregating in a ratio of three awned: one awnless; another segregating in a ratio of one awned: three awnless: and the third, a ratio of fifteen awned: one awnless.

The writer found a case similar to the one last mentioned. The F_1 was fully awned like the awned parent. The F_2 population consisted of four types. One type was fully awned and another fully awnless like the original parents. Of the two new types, one had awns on most of the spikelets, while the other had awns on a few spikelets only, as shown in figure 1. These four types were designated as *fully awned*, *mostly awned*, *rarely awned*, and *fully awnless*, respectively. They occurred in a ratio of

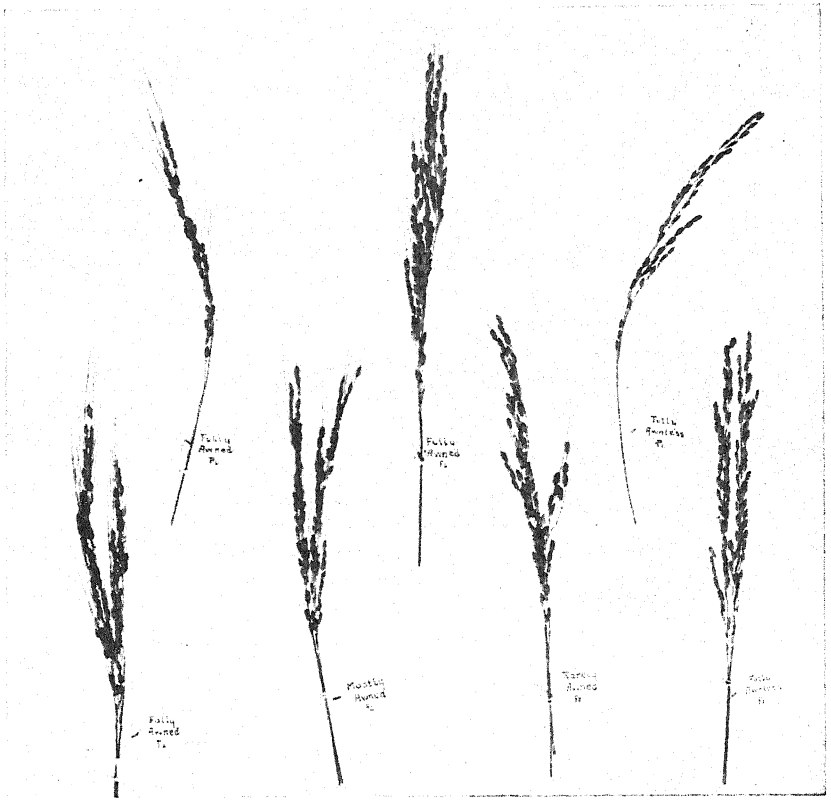


FIGURE 1.—Showing P_1, F_1, and F_2 generations of 101×205, with particular reference to the segregation of the awn character.

12:1:2:1. When all the awned types were classified together, however, the ratio of awned to awnless approaches very closely to 15:1, as shown in table 1.

TABLE 1

Segregation of fifteen awned: one awnless in the F_2 generation of (101×205) D.

PHENOTYPES	OBSERVED	*Calculated 12:1:2:1*	OBSERVED	*Calculated 15:1*	DEVIATION
Fully awned	319	*326.16*			
Mostly awned	29	*27.18*	406	*407.70*	−1.7
Rarely awned	58	*54.36*			
Fully awnless	29	*27.18*	29	*27.18*	1.82

$$\frac{\text{Dev.}}{\text{P.E.}} = \frac{1.82}{3.41} = 0.53$$

These facts are explained on the basis of two pairs of factors $A_{n_1}a_{n_1}$ and $A_{n_2}a_{n_2}$, both being concerned with the production of the awn character. A_{n_2} is considered to be weaker in its action than A_{n_1}. So, A_{n_1} can produce the fully awned type with or without A_{n_2}. A_{n2} in double dose may produce the mostly awned class, but in single dose, it will only produce the rarely awned type. The fully awnless type is due to the double recessive constitution. The interpretation that one of these two factors is weaker in its action than the other is supported by the fact that among the mostly awned and rarely awned types of F_2 plants the frequency of awned spikelets on the early or late panicles of the same plant may vary according to environmental conditions, while among the fully awned or fully awnless types the phenotypic expression is not so easily subject to environmental influence.

These two pairs of factors $A_{n_1}a_{n_1}$ and $A_{n_2}a_{n_2}$ are independent of the glutinous pair, $G_1\,g_1$, as shown in table 2. It is to be noted that among the F_2 population of 434 plants, 349 were non-glutinous and 85 glutinous, showing a deficiency of glutinous grains that is about 3.86 times the probable error.

TABLE 2

Independent segregation between factors $A_{n1}a_{n1}$, $A_{n2}a_{n2}$ and G_1g_1.

PHENOTYPES	OBSERVED	*Calculated 45:3:15:1*	$\frac{(O-C)^2}{C}$
Non-glutinous awned	327	*305.10*	1.57
Glutinous awned	78	*101.70*	5.52
Non-glutinous awnless	22	*20.34*	0.13
Glutinous awnless	7	*6.78*	0.01
	434	*434*	$7.23=X^2$

P=0.0718

The deviation from expectation on the basis of independent segregation is not large, if we take into consideration the significant deficiency of the glutinous plants involved.

Linkage between the T_y t_y pair and the G_1 g_1 pair

In this particular material, when the panicle first emerges from the leaf-sheath, the apiculus, the glumes, and the awn, if present, are green like the other parts of the spikelet. As the spikelet is filled up by the developing grain, the three parts concerned gradually develop color through successive shades from Pale Orange Yellow, Light Orange Yellow up to

Tawny (Ridgway) or even brighter and more glassy than Tawny at maturity. This is designated as the "tawny character," and the absence of it is non-tawny or colorless.

In inheritance, the tawny character is completely dominant over non-tawny in the F_1 generation. In the F_2, the writer obtained a simple 3:1 ratio, as shown in table 3.

TABLE 3

F_2 segregation of tawny non-tawny from the cross (101×205)D.

	TAWNY	NON-TAWNY	TOTAL
Observed	339	99	438
Calculated 3:1	*328.5*	*109.5*	*438*
Deviation		−10.5	

$$\frac{\text{Dev.}}{\text{P.E.}} = \frac{10.5}{6.11} = 1.71$$

Since the tawny color appeared on three parts of the spikelet and no crossing over was noticed in a population of 438 F_2 plants, it is very probable that the tawny character is due to one allelomorphic pair of factors rather than to several different genes completely linked. This allelomorphic pair is designated as T_y t_y.

Breeding data presented in table 4 show that the T_y factor is coupled with the G_1 factor. On the basis of independent segregation, we should

TABLE 4

Coupling between G_1 and T_y
(Gametic ratio r:s:s:r=4.48:1:1:4.48)

	NON-GLUTINOUS TAWNY	NON-GLUTINOUS COLORLESS	GLUTINOUS TAWNY	GLUTINOUS COLORLESS	TOTAL
Observed	309	43	30	58	440
Calculated	*293.11*	*36.39*	*36.39*	*73.44*	*440*
Deviation	15.89	6.61	−6.39	−15.44	

$X^2 = 5.05$
$P = 0.17$

expect 247.5 non-glutinous tawny plants, 82.5 non-glutinous colorless plants, 82.5 glutinous tawny plants, and 27.5 glutinous colorless plants.

But the observed data deviated very widely from the expectation. By EMERSON'S (1916) method, the gametic ratio is found to be:

$$r:s:s:r = 4.48:1:1:4.48$$

From this, the zygotic ratio is calculated. The percentage of crossing over is about 16.59 percent. Considering the significant deficiency of the glutinous plants involved, the calculated ratios are fairly close to the observed ones.

Relation between the pericarp color and the glutinous character

The pericarp color of rice varies from pure white, grey-brown, red to purple. Several investigators notably THOMPSTONE (1915), PARNELL and AYYANGAR (1917), IKENO (1918), NAGAI (1921), and HECTOR (1922), have reported that red and white colors form a simple Mendelian pair segregating in a 3:1 ratio in F_2, red being dominant over white. PARNELL *et al* (1917), however, found a case where red and white segregated in a 9:7 ratio. PARNELL (1922) reported that purple and white also form a Mendelian pair segregating in a 3:1 ratio and that purple×red gave a ratio of 12 purple: 3 red: 1 white in F_2. KATO and ISHIKAWA (1921) found a case where red×white gave a ratio of 9 red:3 yellow:4 white. All these facts seem to indicate that there are two factors concerned with the production of pericarp color.

I found that the Chinese Imperial rice has a red pericarp color which also behaved as a simple Mendelian dominant when crossed with a white variety. This allelomorphic pair is designated as P_{r_1} p_{r_1}. Further data show that P_{r_1} is independent of the factor G_1, as shown in table 5.

TABLE 5

Independent segregation between G_1 and P_{r1} (600×100).

	NON-GLUTINOUS RED PERICARP	NON-GLUTINOUS WHITE PERICARP	GLUTINOUS RED PERICARP	GLUTINOUS WHITE PERICARP	TOTAL
Observed	96	31	37	15	179
Calculated					
9:3:3:1	*100.62*	*33.54*	*33.54*	*11.18*	*179*
Deviation	−4.62	−2.54	3.46	3.82	

In another cross between 4269 and 4957, the former being purple and the latter white, a ratio of 15 colored:1 colorless was obtained, showing that there are two factors concerned. One of these two genes in this case

is presumably the same as P_{r_1} and the other is designated as P_{r_2}. Both of them are independent of the glutinous gene, as shown in table 6.

TABLE 6

Independent segregation between P_{r1} and P_{r2} and g_1 from the cross 4269×4957.

	NON-GLUTINOUS COLORED	NON-GLUTINOUS COLORLESS	GLUTINOUS COLORED	GLUTINOUS COLORLESS	TOTAL
Observed	515	36	147	11	709
Calculated 45:15:3:1	*498.15*	*33.21*	*166.05*	*11.07*	*709*
Deviation	16.85	2.79	−9.05	−0.07	

Inheritance of glume length and its relation with the g_1, p_{r_1}, and p_{r_2} factors

At the base of the spikelet there are two small lance-shaped structures called glumes. In common varieties, the glumes are very short, about one-third as long as the lemma and palea. But there are some varieties in which the glumes are as long as the lemma and palea. In inheritance, PARNELL *et al* (1917) and NAGAI (1921) have reported that the short glume is dominant to the long glume giving a simple 3:1 ratio in F_2.

The writer found a case where the short glume × long glume gave a 15:1 ratio, long glume being recessive, as shown in table 7.

TABLE 7

Segregation for glume length in the cross 4269 (long)×4957 (short).

	SHORT GLUME	LONG GLUME	TOTAL
Observed	674	43	717
Calculated 15:1	*672.15*	*44.81*	*717*
Deviation		−1.81	

$$\frac{\text{Dev.}}{\text{P.E.}} = \frac{1.81}{4.37} = 0.41$$

Since the observed ratio is remarkably close to 15:1, undoubtedly there are two duplicate factors concerned with the production of the glume length. These two pairs of duplicate genes are designated as G_1g_1 and G_2g_2.

Further data clearly indicate that g_1 and g_2 are independent of the glutinous gene g_l, as shown in table 29. The observed data approach the calculated ratios very closely, considering the deficiency of the glutinous plants involved. Furthermore, the duplicate genes G_1 and G_2 have no chromosomal relations with factors P_{r_1} and P_{r_2}, as shown in table 30.

Inheritance of spikelet length and its relation with other characters

The spikelet length varies with different varieties. The variety, 4957, has spikelets varying from 3.5–4.9 mm in length with an average of 4.13 mm. The variety 4269 has spikelets varying from 7.3–10.3 mm in

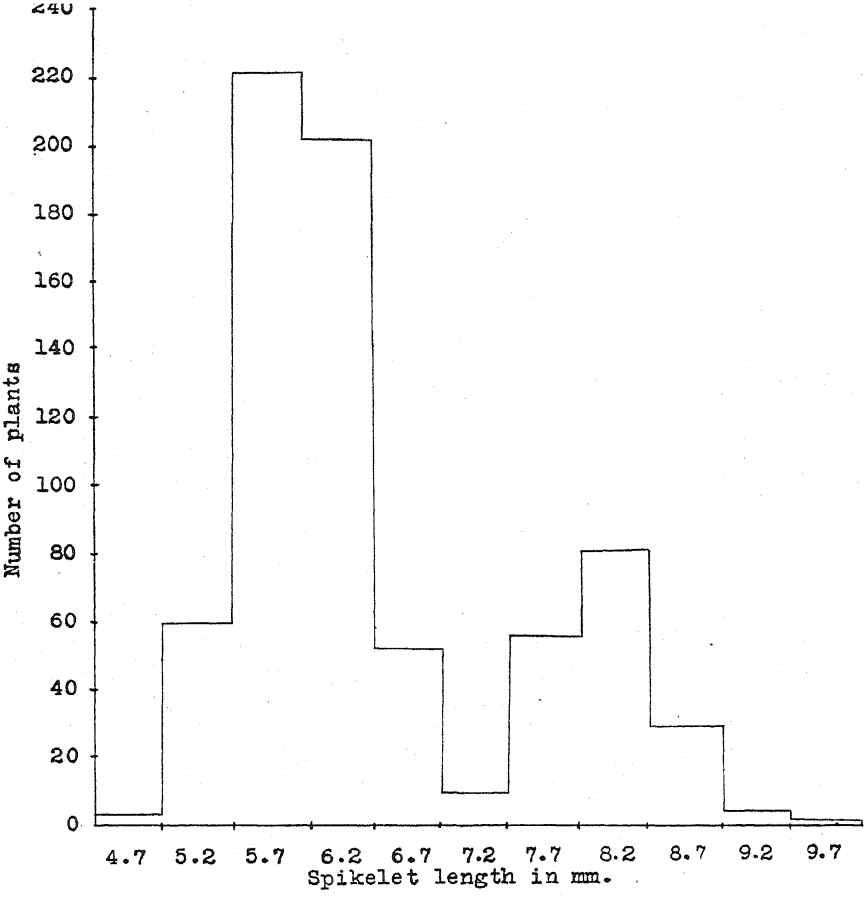


FIGURE 2.—Showing segregation for spikelet length in F_2 of cross 4269×4957.

length with an average of 8.81 mm. The variation within each of these varieties follows a normal frequency curve.

In the cross, 4269×4957, made by the writer at the CROWLEY RICE EXPERIMENT STATION, 1925, ten F_1 plants were obtained, each showing a similar intermediate type of spikelet ranging from 3.9–6.4 mm long with an average of 5.33 mm in length. In the F_2 generation, segregation for the

spikelet length took place, but the spikelets on the same plant were uniform, as expected. Six spikelets taken from the different parts of a panicle in a random fashion were measured and their average length was taken to represent the spikelet length of the plant in question. That this method of sampling actually gives a representative value is proved by the fact that when all the 95 spikelets of a panicle from the plant (Hg_{l_1}) were measured, their average was 5.66 mm approaching very closely to the respective average lengths of several samples of six spikelets each taken at random from that same panicle, namely 5.86, 5.3, 5.75, 5.64, and 5.66. In a population of 718 F_2 plants, the spikelet length ranged from 4.7 to 9.7 mm, as shown in table 8. When the different class values were plotted against the frequency of each class, we obtained a distinctly bimodal curve (figure 2). Since the dividing point of the two groups on the curve

TABLE 8

Showing segregation for spikelet length in F_2 from the cross 4269×4957.

GENERATION	CLASS CENTERS (IN MM) AND FREQUENCIES													NUMBER OF PLANTS	NUMBER OF SPIKELETS
	3.7	4.2	4.7	5.2	5.7	6.2	6.7	7.2	7.7	8.2	8.7	9.2	9.7		
P_1 (4957)	1	3												4	282
P_1 (4269)											4			4	362
F_1				10										10	851
F_2			2	60	222	202	52	9	56	81	29	4	1	718	6 for each plant

is clearly at the class center, 7.2, which is exactly the length of the shortest spikelet of the long parent, and since the longest spikelet of either the short parent or of the F_1 is never over 6.5 mm in length, it appears legitimate to place the nine plants of the class (7.2) in the long group. In so doing, the F_2 population is divided into two phenotypes, one with short spikelets and the other with long spikelets in almost exactly a 3:1 ratio, as shown in table 9.

TABLE 9

Segregation for short and long spikelet in F_2 from 4269×4957.

	SHORT SPIKELET	LONG SPIKELET	TOTAL
Observed	538	180	718
Calculated 3:1	*538.5*	*179.5*	*718*
Deviation		0.5	

The data clearly indicates that the spikelet length, in this material at least, is due to one allelomorphic pair of factors which may be designated as S_p s_p.

The S_p s_p pair of genes are not linked with the G_1 g_1 pair as shown in table 31, nor with P_{r_1} p_{r_1} and P_{r_2} p_{r_2} as shown in table 32.

We have seen, in the preceding pages, that glume length depends upon two independent duplicate factors G_1g_1 and G_2g_2, the short glume being dominant and that the spikelet length depends on one allelomorphic pair of genes, S_p s_p. When the variety, 4957, having short spikelet and short glumes, was crossed with the variety 4269, having long spikelet and long glumes, all F_1 plants had short glumes and intermediate spikelets. In the F_2 generation, two new types occurred in addition to the two grandparental types, as illustrated in figure 3.

The F_2 population consists of 538 plants having short spikelet and short glumes, one plant having short spikelet and long glumes, 134 plants having long spikelet and short glumes, and 45 plants having long spikelet and long glumes, as shown in table 10. On the basis of three independent factors, we should expect the corresponding classes of F_2 plants to be 504.45, 33.63, 168.15, and 11.21, respectively. But this is not the case; the observed data show a great excess of the two parental types. If we assume that one of the glume factors is the same as the spikelet factor, the calculated ratios would be 12:0:3:1, giving the class frequencies 538.50, having short glumes and short spikelet, none having short spikelet with long glumes, 134.625, having long spikelet with short glumes, and 44.875 having long spikelet and long glumes. The expectation on this assumption fits the observed data very well, except that the single plant with short spikelet and long glumes is not accounted for, as shown in table 10.

TABLE 10

Relation between the glume length (15:1) and the spikelet length (3:1).

PROGENIES	SHORT SPIKELET SHORT GLUME	SHORT SPIKELET LONG GLUME	LONG SPIKELET SHORT GLUME	LONG SPIKELET LONG GLUME	TOTAL
4269×4957A	37	0	3	6	
" B	48	0	11	4	
" C	52	1 (CSTG)	13	3	
" D	58	0	14	5	
" E	45	0	6	5	
" F	76	0	22	8	
" G	68	0	16	5	
" H	4	0	4	0	
" I	73	0	18	6	
" J	77	0	27	3	
Observed	538	1	134	45	718
Independent 45:3:15:1	504.45	33.63	168.15	11.21	717.44
Independent 12:0:3:1	538.50	0	134.625	44.875	718

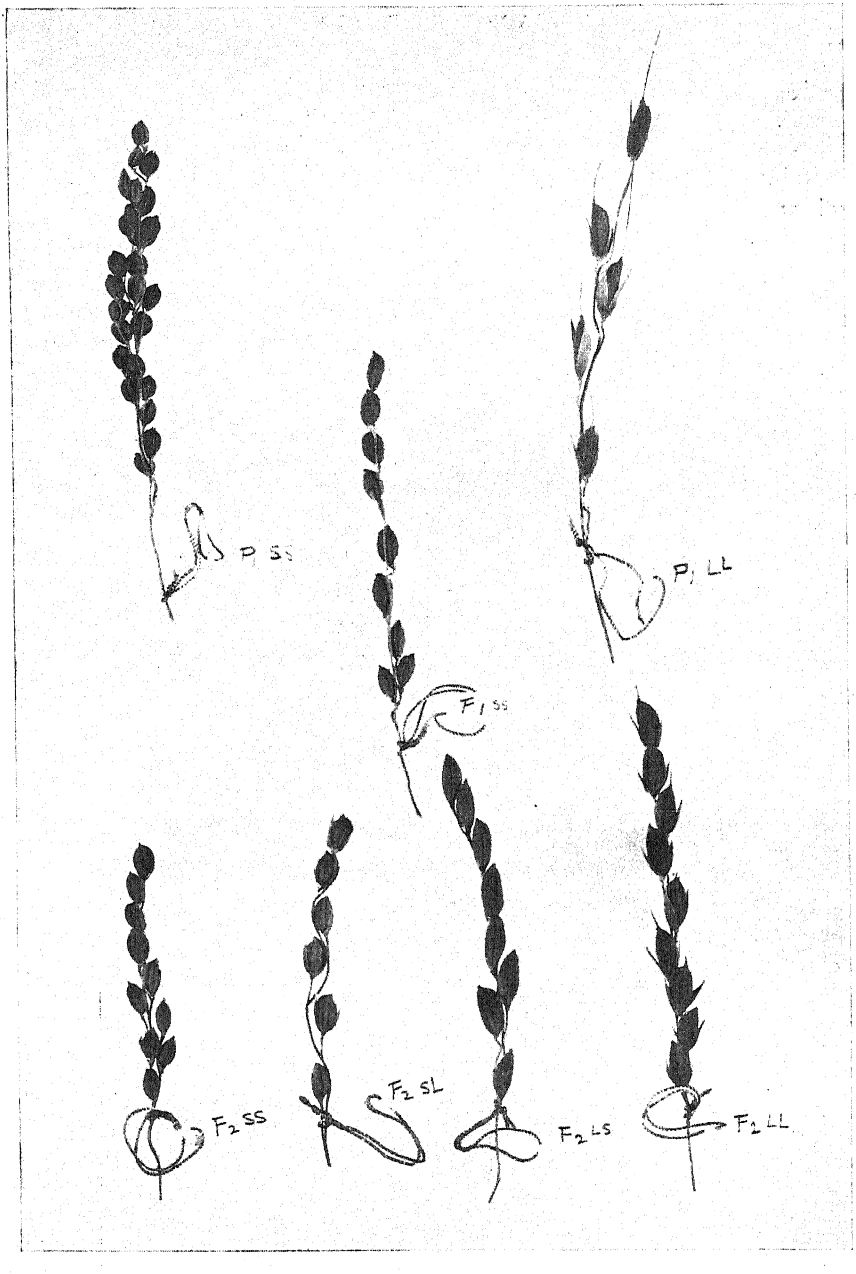


FIGURE 3.—P_1 *ss* = The paternal parent having short spikelet and short glumes. P_1 *LL* = The maternal parent, having long spikelet and long glumes. F_1 *ss* = Hybrid, having short or intermediate spikelet and short glumes. F_2 *ss* = One of 538 F_2 plants, having short spikelet and short glumes. F_2 *sL* = The only F_2 plant which had short spikelets and long glumes. This combination is a result of crossing over. F_2 *Ls* = One of the 134 F_2 plants having long spikelets and short glumes. F_2 *LL* = One of the 45 plants having long spikelets and long glumes.

The same difficulty occurs if we assume complete linkage between one of the glume factors and the spikelet factor.

So far as the evidence goes, the best explanation, in the writer's opinion, may be obtained on the basis of close linkage between one of the duplicate factors for glume length and the factor for spikelet length. Here, it may be arbitrarily assumed that g_2 is closely linked with s_p. On this assumption, we can calculate the gametic ratio.

If only the factors G_1g_1 and $S_p\ s_p$ had been concerned in this cross, the F_2 distribution would be represented by the general formula,

$$\frac{a}{3r^2+2(s^2+2rs)}:\frac{b}{s^2+2rs}:\frac{c}{s^2+2rs}:\frac{d}{r^2} \qquad (1)$$

Where $r:s:s:r$ is any gametic series and a, b, c, and d are the phenotypes, G_1S_p, G_1s_p, g_1S_p, and g_1s_p, respectively.

But when the G_2g_2 pair of genes is also involved, as is the case in this cross, r = the non-crossover gametes $G_1G_2S_p$, $g_1G_2S_p$, $G_1g_2s_p$, and $g_1g_2s_p$; and s = crossover gametes $G_1G_2s_p$, $g_1G_2s_p$, $G_1g_2S_p$, and $g_1g_2S_p$. Combinations of these gametes in all possible ways grouped according to phenotypes may be represented by the general formula,

$$\frac{a}{12r^2+11(s^2+2rs)}:\frac{b}{3r^2+4(s^2+2rs)}:\frac{c}{s^2+2rs}:\frac{d}{r^2} \qquad (2)$$

From formula (2), we get the following four equations:

$a = 12r^2+11(s^2+2rs)$ = Short spikelet and short glumes
$b = 3r^2+4(s^2+2rs)$ = Long spikelet and short glumes
$c = 2rs+s^2$ = Short spikelet and long glumes
$d = r^2$ = Long spikelet and long glumes

From these four equations, we can determine the gametic ratio directly from the observed zygotic series. Thus, we get $a+b+c+d=16r^2+16c$

$$16r^2 = a+b+d-15c$$

$$r^2 = \frac{a+b+d-15c}{16}$$

$$r = 0.25\sqrt{a+b+d-15c} \qquad (3)$$

Also,

$$16(s^2+2rs) = a+b+c-15r^2$$

$$s^2+2rs = \frac{a+b+c-15r^2}{16}$$

Adding r^2 (or d) to both sides, we get,

$$r^2+2rs+s^2=\frac{a+b+c+d}{16}$$

$$r+s=0.25\sqrt{a+b+c+d}$$

$$s=0.25\sqrt{a+b+c+d}-r \qquad (4)$$

By substituting the observed data of the four phenotypes for a, b, c, and d, in the formulae (3) and (4), we get,

$$r=6.6225$$
$$s=0.0750$$

If we take $s=1$, the ratio of $\frac{r}{s}=\frac{88.3}{1}$

By substituting the values of r and s in the four equations, we obtain the expected frequencies of the four phenotypes, which fit the observed data unusually closely, as shown in table 11.

TABLE 11

Showing coupling between g_2 and s_p (crossover = 1.11 percent).

	SHORT SPIKELET SHORT GLUMES	LONG SPIKELET SHORT GLUMES	SHORT SPIKELET LONG GLUMES	LONG SPIKELET LONG GLUMES	TOTAL
Observed	538	134	1	45	718
Calculated (linkage)	*537.5*	*135.63*	*0.99*	*43.87*	
Deviation	0.5	−1.63	0.01	1.13	

$X^2=0.0533$. When $X^2=1$, $P=.801253$

Since this is a coupling phase, the crossover between g_2 and s_p will be,

$$\frac{s}{r-s}\times 100=1.11 \text{ percent.}$$

Inheritance of the apiculus color and its relation with other characters

The apex of the lemma and palea is colored in many varieties. This localized color spot at the upper tip of the spikelet is here spoken of as the apiculus color. The inheritance of this character has been studied by several investigators. HECTOR obtained a ratio of three colored apiculus to one colorless in 1913 and another ratio of 27:37 in 1916. Besides, HECTOR (1922) reported two new conditions, one of which segregated in a 9:7 ratio and the other in a 15:1 ratio.

In crosses between different varieties, the writer obtained various ratios in the F_2 generations, namely, 3:1, 9:7, 15:1, 27:37, and 162:94, indicating that there are at least four genetic factors responsible for the production of the apiculus color. The data are presented in table 12.

TABLE 12

Showing segregation of colored and colorless apiculus in different ratios in F_2 populations of different crosses.

	CROSSES AND THE PARENTAL TYPES	F_1	F_2 SEGREGATION		CLOSEST RATIO	EXPECTED F_2		DEVIATION	SYMBOLS
			Colored	Colorless		Colored	Colorless		
1	Colorless×colored 300×400	Colored	77	65	9:7	79.83	62.09	2.91	CC A_{p_1}, A_{p_2}
2	Colorless×colorless 300×600	Colored	356	499	27:37	360.45	493.95	5.05	Cc A_{p_1}, A_{p_3}
3	Colorless×colored 600×400	Colored	330	190	162:94	328.86	190.82	−0.82	A_{p_1}, Cc A_{p_2}, A_{p_3}
4	Colored×colorless 800×600	Colored	255	22	15:1	259.65	17.31	4.69	A_{p_5}, A_{p_6}
5	Colorless×colored 4269×4957	Colored	567	176	3:1	557.25	183.75	7.75	A_{p_4}

That variety 300, though colorless, actually carries some factor is shown by the fact that when it was crossed with another colorless variety 600 (see cross No. 2), the F_1 had colored apiculus. In the F_2, a ratio of 27:37 was observed, showing at least three factors were involved. This situation may be explained, if the two parental types have the following genetic constitutions:

$300 = g_l\ g_l\ CC\ a_{p_1}\ a_{p_1}\ a_{p_2}\ a_{p_2}\ a_{p_3}\ a_{p_3}$ (glutinous colorless)

$600 = G_l\ G_l\ cc\ A_{p_1}\ A_{p_1}\ A_{p_2}\ A_{p_2}\ A_{p_3}\ A_{p_3}$ (non-glutinous colorless

Where A_{p_1}, A_{p_3} and C are complimentary factors for apiculus color.

In cross No. 1 (300×400) the 9:7 ratio clearly indicates that two factors are concerned in the production of the apiculus color, these being designated as A_{p_1} and A_{p_2}. The situation can be explained by assuming the genetic constitutions of the parents as follows:

Parent $300 = g_l\ g_l\ CC\ a_{p_1}\ a_{p_1}\ a_{p_2}\ a_{p_2}$

Parent $400 = G_l\ G_l\ CC\ A_{p_1}\ A_{p_1}\ A_{p_2}\ A_{p_2}$

Where C = chromogen, and A_{p_1} and A_{p_2} are complementary for the apiculus color.

That the variety 600 actually carries at least one apiculus factor differing from those in the variety 400 is proved by cross No. 3, which gave a ratio of 162:94, showing that four factors are involved. The situation may be explained on the bases of the foregoing genetic constitutions assumed for varieties 400 and 600, respectively.

Cross No. 4 gave a ratio of 15:1, showing that there are two factors involved, each of them alone producing apiculus color. These factors may be designated as A_{p_5} and A_{p_6}. As no further crosses were made, the assumption must be considered as a tentative one.

Cross No. 5 gave a simple 3:1 ratio, showing that only one factor pair was involved. This factor pair is different from A_{p_1}, A_{p_2}, and A_{p_3}, in that none of the latter alone can produce the apiculus color. Furthermore, this new factor is linked with the glutinous gene as will be shown later. This gene is designated as A_{p_4}. It may be that one of the two duplicate factors involved in cross No. 4 is the same as A_{p_4}.

Factors A_{p_1} and A_{p_2} are independent of the glutinous gene as shown in table 13.

TABLE 13

Showing independent segregation between factors A_{p_1}, A_{p_2} and g_l (307×410).

	COLORED APEX NON-GLUTINOUS	COLORLESS APEX NON-GLUTINOUS	COLORED APEX GLUTINOUS	COLORLESS APEX GLUTINOUS	TOTAL
Observed	65	51	12	14	142
Calculated 27:21:9:7	*59.67*	*46.41*	*19.89*	*15.47*	*142*
Deviation	5.33	4.59	−7.89	−1.47	

As mentioned above, A_{p_4} is linked with the glutinous gene as shown in table 14. On the independent Mendelian basis, the expected frequencies should be 417.87 colored non-glutinous, 139.29 colored glutinous, and 46.43 colorless glutinous. But this is far from the observed data which clearly indicate coupling between a_{p_4} and g_l. On the latter basis, the

TABLE 14

Showing coupling between a_{p_4} and g_l from F_2 of 4269×4957.

	COLORED APEX NON-GLUTINOUS	COLORLESS APEX NON-GLUTINOUS	COLORED APEX GLUTINOUS	COLORLESS APEX GLUTINOUS	TOTAL
Observed	491	90	76	86	743
Calculated	*474.17*	*83.16*	*83.16*	*102.62*	*743*
Deviation	16.83	6.84	−7.16	−16.62	

$X^2=4.46.\quad P=0.22.$

gametic ratio is found from EMERSON'S (1916) formula, $r = 10.13$, and $s = 3.5$. The crossover is, therefore, about 22.34 percent.

The large deviation is clearly due to the deficiency of the glutinous plants, the latter being 2.98 times the probable error.

Further data show that A_{p4} is independent of the factors P_{r_1} and P_{r_2}, G_1 and G_2, and S_p, as is seen in tables 33, 34, and 35 respectively.

Relation between stigma color and other characters

In some varieties, the stigma is colorless, and in others it is colored with an intensity varying from pale red to dark purple. In inheritance, colored and colorless stigma segregate in different ratios according to the material used. HECTOR (1916, 1922) reported cases of 3:1, 9:7, 27:37, and 81:175 ratios, showing that there are at least five factors responsible for the production of stigma color.

The writer obtained two cases, one segregating in a 3:1 ratio, and the other, 9:7, as shown in table 15.

TABLE 15

Showing segregation of stigma color.

CASE	CROSSES COLORLESS × PURPLE	F_1	F_2 SEGREGATION		CLOSEST RATIO	F_2 EXPECTED		DEVIATION	DEV. / P. E.	SYMBOLS
			Colored	Colorless		Colored	Colorless			
1	307×410	Colored	101	41	3:1	106.5	35.5	5.5	1.58	
2	4269×4957 (ABFGI)	Colored	242	179	9:7	236.79	184.17	5.17	0.75	S_{a_1} S_{a_2}

In the first case, the factor is probably the same as A_{p_1} (or A_{p_2}), because in the same F_2 population arising from the same cross, the apiculus color alone segregated in a 9:7 ratio (see table 12) whereas a 9:3:4 ratio was observed when both apiculus and stigma were considered at the same time, as shown in table 16.

TABLE 16

Showing F_2 segregation for apex and stigma color (307×410).

	COLORED STIGMA COLORED APEX	COLORED STIGMA COLORLESS APEX	COLORLESS STIGMA COLORED APEX	COLORLESS STIGMA COLORLESS APEX	TOTAL
Observed	77	24	0	32	142
Calculated 9:3:4	*79.83*	*26.61*	*0*	*35.48*	*142*
Deviation	−2.83	−2.61	0	3.48	

In the second case, there are clearly two separate factors for the production of the stigma color. These are designated as S_{a_1} and S_{a_2}. They are complementary to each other. One of them is linked with the glutinous gene, g_l. For the sake of convenience, the linkage may be arbitrarily assumed to be between s_{a_1} and g_l. The data are presented in table 17.

TABLE 17

Showing linkage between s_{a_1} and g_l in F_2 generation from the cross 4269×4957.

	NON-GLUTINOUS PURPLE STIGMA	NON-GLUTINOUS COLORLESS STIGMA	GLUTINOUS PURPLE STIGMA	GLUTINOUS COLORLESS STIGMA	TOTAL
Observed	210	100	32	79	421
Independent 27:21:9:7	177.39	137.97	59.13	45.99	421
Linkage	210.23	105.45	26.53	78.69	421
Deviation	−0.23	−5.45	5.47	0.31	

$$X^2=1.4094. \quad P=0.707564.$$

BRUNSON'S (1924) modified formulae

$$r=\sqrt{\frac{(AB+3ab)-(Ab+aB)}{18}}$$

$$s=\frac{1}{4}\sqrt{AB+Ab+aB+ab}-r$$

were used in calculating the intensity of the linkage between s_{a_1} and g_l. The gametic ratio is $r=4.18$ and $s=0.95$. Since this is a coupling phase, the percentage of crossing over is obtained from the formula $\frac{s}{r+s}$, namely 18.51 percent.

Data from the same cross show factors s_{a_1} and s_{a_2} are independent of p_{r_1}, p_{r_2}, g_1, g_2 and sp as shown in tables 36, 37, and 38.

The exact relation between the stigma color and the apiculus color in this particular cross is not determined. As described above, a_{p_4} is coupled with g_l, giving about 22.34 percent crossing over; and s_{a_1} is also linked with g_l, giving 18.51 percent crossing over. When the apiculus color and the stigma color were involved at the same time, a new situation arose, as shown in table 18.

The zygotic ratios calculated on the basis of three independent factors do not fit the observed ratio at all. There are, then, only two alternative explanations for the situation, namely, complete linkage between a_{p_4} and s_{a_1}, or a_{p_4} being the same factor as s_{a_1}. Since both alternatives give identical

zygotic ratio (9:3:0:4), it is impossible to decide which alternative is correct. It may be mentioned, however, that one plant (FSt 68) which is not counted in table 18 had purple stigma with a doubtful apiculus color, because the latter was under a question mark (?) in the original notebook.

TABLE 18

Relation between a_{p_4} and s_{a_1}.

	COLORED APEX PURPLE STIGMA	COLORED APEX COLORLESS STIGMA	COLORLESS APEX PURPLE STIGMA	COLORLESS APEX COLORLESS STIGMA	TOTAL
Observed	243	64	1 (FSt 68?)	117	424
Calculated 27:21:9:7	*178.74*	*139.02*	*59.58*	*46.34*	*424*
Complete linkage 9:3:0:4	238.5	79.5	0	106.0	424
Deviation	4.5	−15.5	0	11.0	

Although a_{p_4} and s_{a_1} have different crossover values with the glutinous gene, the difference is only 3.83 percent. Since both alternatives are possible, the question must be left open for the present.

Relation between the leaf-sheath color and other characters

The leaf-sheath color when present may be either self red or purple, or merely consisting of colored stripes varying in intensity. There are several genetic factors responsible for its production. PARNELL (1917) found a case where colored and colorless leaf-sheath segregated in a simple 3:1 ratio. HECTOR (1916, 1922) reported four cases segregating in 3:1, 9:7 27:37, and 15:1, respectively.

The writer has obtained two kinds of ratios, namely, 9:7 and 15:1, as shown in table 19.

TABLE 19

Showing F_2 segregation for leaf-sheath color.

CASE	CROSSES	F_1	F_2 SEGREGATION		CLOSEST RATIO	*Expected*		DEVIATION	SYMBOLS
			Colored	Colorless		Colored	Colorless		
1	4269×4957 (colorless×colored)	Colored	204	139	9:7	*192.87*	*147.01*	−8.01	Ls_1 Ls_2
2	800b_5×625 (colored×colorless)	Colored	266	14	15:1	*262.5*	*17.5*	−2.05	Ls_3 Ls_4

These data indicate that there are at least four factors which are concerned with the production of leaf-sheath color. In the first case, there must be two complementary factors, whose presence is necessary for the expression of color. These factors are designated as L_{s_1} and L_{s_2}. In the

second case, there must be two duplicate factors each of which alone can produce color. These are designated as L_{s_3} and L_{s_4}.

In the first cross, one of the complementary factors is linked with the glutinous gene. The linkage may be arbitrarily assumed to be between l_{s_1} and g_l. The gametic ratio is calculated by BRUNSON'S formulae,

$$r=\sqrt{\frac{AB+3ab-(Ab+aB)}{18}}=3.73$$

$$s=\frac{1}{4}\sqrt{AB+Ab+aB+ab}-r=0.90$$

The crossing over $=\frac{s}{r+s}\times 100=19.43$ percent. By substituting the values of r and s in the following equations, we get the zygotic ratios,

$AB=9r^2+12rs+6s^2=$ colored sheath non-glutinous
$Ab=\quad 6rs+3s^2=$ colored sheath glutinous
$aB=3r^2+12rs+6s^2=$ colorless sheath non-glutinous
$ab=4r^2+\ 2rs+\ s^2=$ colorless sheath glutinous

The calculated zygotic ratios fit the observed ratios very well considering the deficiency of glutinous plants, as shown in table 20.

TABLE 20

Showing coupling between ls_1 and g_l.

	COLORED SHEATH NON-GLUTINOUS	COLORED SHEATH GLUTINOUS	COLORLESS SHEATH NON-GLUTINOUS	COLORLESS SHEATH GLUTINOUS	TOTAL
Observed	179	25	80	59	343
Calculated 27:9:21:7	*144.72*	*48.24*	*112.56*	*37.52*	*343*
Linkage (19.43% crossing over)	170.36	22.57	88.88	63.18	343
Deviation	8.64	2.43	−8.88	−4.18	

Data obtained from the same cross indicate that l_{s_1}, l_{s_2} are independent of the factors p_{r_1}, p_{r_2}, g_1, g_2, and s_p, as shown in tables 39, 40 and 41.

l_{s_1} is closely linked with a_{p_4} as shown in table 21. Since a_{p_4} is coupled with g_l, giving 22.34 percent crossing over, and since l_{s_1} is also linked with g_1, giving 19.43 percent crossing over, the order of the three genes on the glutinous chromosome would appear to be,

$$a_{p_4}\quad l_{s_1}\qquad \overset{22.34\text{ percent}}{\overbrace{\qquad\qquad}}\qquad g_l$$
$$\qquad\qquad\quad 19.43\text{ percent}$$

Linkage between stigma color and leaf-sheath color

As described above, in the F_2 generation of the cross, 4269×4957, the stigma color alone segregated in a ratio of 9 colored:7 colorless, and the leaf-sheath color also segregated in a 9:7 ratio. One of the two complemen-

TABLE 21

Showing linkage between a_{p_4} and l_{s_1} in F_2 (4269×4957).

	COLORED APICULUS PURPLE SHEATH	COLORED APICULUS COLORLESS SHEATH	COLORLESS APEX PURPLE SHEATH	COLORLESS APEX COLORLESS SHEATH	TOTAL
Observed	205	43	(BSt$_3$) 1	98	347
Expected 27:21:9:7	*146.34*	*113.82*	*48.78*	*37.94*	*346.88*
Deviation	58.66	−50.82	−47.78	60.06	

tary factors for stigma color is linked with the glutinous gene, giving 18.71 percent crossing over. One of the two complementary genes for the production of the leaf-sheath color is also linked with g_l, giving 19:43 percent crossing over. When the stigma color and the leaf-sheath color are considered at the same time, a very close linkage between the two characters is revealed, as shown in table 22a, where the calculated zygotic ratios on the basis of four independent factors are shown to be very far from the observed frequencies.

TABLE 22a

Showing close linkage between the stigma color and the leaf-sheath color.

PROGENIES	PURPLE STIGMA PURPLE SHEATH	PURPLE STIGMA COLORLESS SHEATH	COLORLESS STIGMA PURPLE SHEATH	COLORLESS STIGMA COLORLESS SHEATH	TOTAL
4269×4957B	34	2	4	23	
4269×4957D	46	3	2	29	
4269×4957F	52	4	3	44	
4269×4957I	57	0	6	37	
Observed	189	9	15	133	346
Calculated on 4 independent factors	*109.35*	*85.05*	*85.05*	*66.15*	
3 independent factors	*145.97*	*48.66*	*48.66*	*102.72*	

Since the stigma and the leaf-sheath give about the same percentage of crossing over with the glutinous gene, the difference being 0.72 percent, it is possible that the two characters have one factor in common. Most probably s_{a_1} is the same factor as l_{s_1}. For the sake of simplicity, this common factor is designated as A. Until further data demonstrate that the s_{a_1} and l_{s_1} are actually two separate entities, factor A will be considered

as a common gene which has a manifold effect conditioning stigma color as well as leaf-sheath color. On this hypothesis, then, A is complementary with S_{a_2} for stigma color and A is also complementary with L_{s_2} for leaf-sheath color.

Since the calculated ratios on the basis that A, S_{a_2} and L_{s_2} are independent factors do not fit the observed frequencies, S_{a_2} and L_{s_2} must be coupled. The situtation may be represented by the following diagram:

$$\begin{array}{|c|c|} A & a \\ Gl & g_1 \end{array} \qquad \begin{array}{|c|c|} s_{a_2} & S_{a_2} \\ l_{s_2} & L_{s_2} \end{array}$$

If $r:s:s:r$ represents any gametic series between the two linked factors, s_{a_2} and l_{s_2}, the non-crossover classes may be represented by the following equations:

$$\begin{aligned} A\ S_{a_2}\ L_{s_2} &= 3\,[3r^2+2(s^2+2rs)\,] = 9r^2+12rs+6s^2 \\ a\ S_{a_2}\ L_{s_2} &= \quad 3r^2+2(s^2+2rs) \quad = 3r^2+\ 4rs+2s^2 \\ A\ \ s_{a_2}\ \ l_{s_2} &= \ (3r^2) \qquad\qquad\quad = 3r^2 \\ a\ \ s_{a_2}\ \ l_{s_2} &= \ (\ r^2) \qquad\qquad\quad = \ r^2 \end{aligned}$$

On the other hand, the cross over classes may be represented as follows

$$\begin{aligned} A\ \ s_{a_2}\ L_{s_2} &= 3(s^2+2rs) = 6rs+3s^2 \\ A\ S_{a_2}\ \ l_{s_2} &= 3(s^2+2rs) = 6rs+3s^2 \\ a\ S_{a_2}\ \ l_{s_2} &= \ (s^2+2rs) = 2rs+s^2 \\ a\ \ s_{a_2}\ L_{s_2} &= \ (s^2+2rs) = 2rs+s^2 \end{aligned}$$

Since either stigma color or leaf-sheath color needs two complementary factors ($A\ S_{a_2}$ or $A\ L_{s_2}$) for its expression, the five classes ($a\ S_{a_2}\ L_{s_2}$, $A\ s_{a_2}\ l_{s_2}$ $a\ s_{a_2}\ l_{s_2}$, $a\ S_{a_2}\ l_{s_2}$, $a\ s_{a_2}\ L_{s_2}$) are thrown into the same phenotypic group By this regrouping, the four phenotypic classes are represented by the four equations:

$$\begin{aligned} XY &= 9r^2+12rs+6s^2 && \text{Purple stigma and purple sheath} \\ Xy &= \quad 6rs+3s^2 && \text{Purple stigma and colorless sheath} \\ xY &= \quad 6rs+3s^2 && \text{Colorless stigma and purple sheath} \\ xy &= 7r^2+\ 8rs+rs^2 && \text{Colorless stigma and colorless sheath} \end{aligned}$$

Where X = purple stigma, x = colorless stigma, Y = purple sheath and y = colorless sheath. From these equations, we get:

$$XY+xy = 16r^2+20rs+10s^2 \qquad \text{(A)}$$

$$Xy+xY = \quad 12rs+\ 6s^2 \qquad \text{(B)}$$

Dividing both (A) and (B) by 2, we get

$$\frac{XY+xy}{2}=8r^2-10rs+5s^2 \qquad (C)$$

$$\frac{Xy+xY}{2}=\qquad 6rs+3s^2 \qquad (D)$$

Multiplying (C) by 3, and (D) by 5, we get

$$24r^2=\frac{3}{2}(XY+xy)-\frac{5}{2}(Xy+xY)$$

$$r=\sqrt{\frac{\frac{3}{2}(XY+xy)-\frac{5}{2}(Xy+xY)}{24}} \qquad (1)$$

Also,

$$XY+Xy+xY+xy=16(r^2+2rs+s^2)$$

Taking the square roots, we get

$$4(r+s)=\sqrt{XY+Xy+xY+xy}$$

$$s=\tfrac{1}{4}\sqrt{XY+Xy+xY+xy}-r \qquad (2)$$

From (1) and (2), we can calculate the gametic ratio, this being:

$$r=4.19$$
$$s=0.46$$

Since this is a coupling case, the percentage of crossing over =

$$\frac{0.46}{4.19+0.46}\times 100=9.8 \text{ percent}$$

Substituting the calculated values of r and s in the four original equations representing the four F_2 phenotypic classes, we get the frequencies quite similar to the observed ones, as shown in table 22b:

TABLE 22b

Showing close linkage between sa_2 and ls_2.

	PURPLE STIGMA PURPLE LEAF-SHEATH	PURPLE STIGMA COLORLESS SHEATH	COLORLESS STIGMA PURPLE SHEATH	COLORLESS STIGMA COLORLESS SHEATH
Observed	189	9	15	133
Calculated	*182.68*	*12.19*	*12.19*	*138.88*
Deviation	6.32	−3.19	2.81	−5.88

$$X^2=1.947$$
$$P=.584536$$

Relation between ligule color and other characters

The ligule is a structure that projects out at the juncture of the leaf-blade and the leaf-sheath. In some varieties, this structure has a deep purple color. In inheritance, the ligule color depends on several factors for its expression. HECTOR (1922) reported two cases, one segregating in 9:7 ratio and the other segregating in 27:37 ratio.

The writer has obtained a ratio of 27 purple: 37 green in the cross, 4269×4957, as shown in table 23.

TABLE 23

Showing segregation for ligule color.

	LIGULE PURPLE	LIGULE GREEN	TOTAL
Observed	109	148	257
Calculated 27:37	*108.41*	*148.55*	*257*
Deviation	0.59	−0.55	

The data clearly show that three factors are concerned in the production of ligule color in this case. These factors are designated as l_{g_1}, l_{g_2}, and l_{g_3}. These three genes are probably independent of the factors g_l, g_1, g_2, and s_p, as shown in tables 24, 42, and 43.

TABLE 24

Showing independent segregation of g_l, l_{g_1}, l_{g_2}, and l_{g_3}.

PROGENIES	NON-GLUTINOUS PURPLE LIGULE	NON-GLUTINOUS GREEN LIGULE	GLUTINOUS PURPLE LIGULE	GLUTINOUS GREEN LIGULE	TOTAL
4269×4957A	21	15	2	9	
4269×4957B	18	26	4	15	
4269×4957C	33	27	4	5	
4269×4957D	23	42	4	9	
Observed	95	110	14	38	257
Calculated 81:111:27:37	*81.32*	111.*44*	*27.11*	*37.15*	
Deviation	13.68	−1.44	−13.11	0.85	

Relation between ligule color and pericarp color

As mentioned above, the pericarp color in this cross is due to two duplicate factors which have been designated as P_{r_1} and P_{r_2}. When the ligule color and the pericarp color are considered at the same time, a new situation appears, as shown in table 25.

As shown in table 25, there is only one individual in the class "purple ligule and colorless pericarp" from four progenies. It is possible that some of the ligule factors are closely linked with the pericarp genes, and the rare occurrence of this class is due to linkage and the small population.

TABLE 25

Showing relation between p_{r1}, p_{r2}, l_{g1}, l_{g2}, and l_{g3}.

PROGENIES	PURPLE LIGULE COLORED PERICARP	PURPLE LIGULE COLORLESS PERICARP	GREEN LIGULE COLORED PERICARP	GREEN LIGULE COLORLESS PERICARP	TOTAL
4269×4957A	21	(Agl_5) 1	20	4	
4269×4957B	22	0	36	5	
4269×4957C	38	0	27	3	
4269×4957D	26	0	48	4	
Observed	107	1	131	16	255
Calculated 108:0:132:16	*108*	*0*	*132*	*16*	
Deviation	−1	1	−1	0	

On the other hand, it is equaly possible that one of the ligule genes is the same as one of the pericarp factors, and the single individual may be due to contamination. Indeed, the calculated ratios (108:0:132:16) on the latter basis fit the observed ratios remarkably well. However, the question must be left open for the present. Earlier investigations (HECTOR 1922) have shown a similar situation.

Relation of hull color to other characters

The term "hull" here used includes the lemma and palea which enclose the grain within. There are different colors either extended entirely over the hull such as "dark gold, ripening gold, and ripening straw," or restricted to certain portions of the hull, such as "dark furrows, piebald gold, tipped gold, patchy gold, mottled gold, and granular furrows" (PARNELL 1922). The character with which we are immediately concerned here is the ripening black color. The hull is first green as usual, but when the grains reach maturity, the hull turns black. PARNELL (1917) reported two cases of a similar condition, one segregating in a 3:1 ratio and the other 9:7.

The writer crossed two non-black varieties ($800b_5 \times 625$), the F_1 spikelets at first had green hull which later changed black or sooty black (Ridgway). The F_2 plants segregated in a ratio of approximately 9 black :7 non-black, as shown in table 26, where the data for this and other characters involved in the same cross ($800b_5 \times 625$) are presented together.

TABLE 26

Showing segregation for colors of hull, internode, leaf-sheath, apiculus, and pericarp.

	DESCRIPTION	F_2 SEGREGATION		CLOSEST RATIO	F_2 EXPECTATION		DEV. / P. E.	SYMBOLS
Hull	Black *versus* non-black	115	103	9:7	122.76	95.48	1.52	h_1 h_2
Internode	Colored *versus* colorless	142	117	9:7	145.13	112.88	0.76	In_1 In_2
Leaf-sheath	Purple *versus* colorless	266	14	15:1	262.5	17.5	0.91	ls_3 ls_4
Apiculus	Colored *versus* colorless	255	22	15:1	259.65	17.31	1.72	ap_5 ap_6
Pericarp	Colored *versus* colorless	199	19	15:1	203.42	13.56	2.25	pr_1 pr_2

The data clearly show that there are two complementary factors for the production of the black hull. These are designated as H_1 and H_2. Both these genes are independent of the factors I_{n_1}, I_{n_2}, L_{s_3}, L_{s_4}, A_{p5}, A_{p6}, P_{r_1}, and P_{r2}, as shown in tables 27, 28, 44, and 45.

TABLE 27

Showing independent segregation between h_1, h_2, i_{n_1}, and i_{n_2}.

	BLACK HULL COLORED INTERNODE	BLACK HULL COLORLESS INTERNODE	NON-BLACK COLORED INTERNODE	NON-BLACK COLORLESS INTERNODE	TOTAL
Observed	63	47	44	57	211
Calculated 81:63:63:49	*66.74*	*51.91*	*51.91*	*40.38*	*210.94*
Deviation	−3.74	−4.91	−7.91	16.62	

The large deviation of the observed frequencies may be due to the small population, because at least 256 individuals are necessary to make the ratios barely even.

TABLE 28

Showing independent segregation between h_1, h_2, l_{s_3}, and l_{s_4}.

	BLACK HULL PURPLE SHEATH	NON-BLACK HULL PURPLE SHEATH	BLACK HULL COLORLESS SHEATH	COLORLESS HULL COLORLESS SHEATH	TOTAL
Observed	111	97	3	6	217
Calculated 135:105:9:7	*114.75*	*89.25*	*7.65*	*5.95*	*217*
Deviation	−3.75	7.75	−4.65	0.05	

DISCUSSION OF RESULTS

In the foregoing pages, data have been presented to show the behavior and chromosomal relations of twenty-five or more genetic factors affecting one or another part of the plant. To facilitate further discussion, we may outline the established genes and their behavior as follows:

FACTORS	CHARACTERS FOR WHICH THE FACTORS ARE RESPONSIBLE	RATIOS
G_1 g_1	The non-glutinous and glutinous pair	3:1
A_{n_1} a_{n_1} A_{n_2} a_{n_2}	Duplicate factors for the production of awns Independent of G_1 g_1 pair	15:1
T_y t_y	For the tawny color on the awn, apex and glumes Linked with G_1 g_1—crossover = 16.59 percent	3:1
P_{r_1} p_{r_1} P_{r_2} p_{r_2}	Duplicate factors for pericarp color Independent of g_1	15:1
G_1 g_1 G_2 g_2	Duplicate factors for the glume length Independent of g_{l_1}, p_{r_1} and p_{r_2}	15:1
S_p s_p	Factor pair for spikelet length Independent of g_{l_1}, p_{r_1}, p_{r_2} and g_1 Closely linked with g_2—crossing over 1.11 percent	3:1
A_{p_1} a_{p_1} A_{p_2} a_{p_2}	Complementary factors for apiculus color Independent of g_1	9:7
A_{p_3} a_{p_3}	A third factor for apiculus color	(27:37)
A_{p_4} a_{p_4}	Factor for apiculus color Linked with g_1—crossing over = 22.34 percent Independent of p_{r_1}, p_{r_2}, g_1, and s_p	3:1
A_{p_5} a_{p_5} A_{p_6} a_{p_6}	Duplicate factors for the apiculus color	15:1
S_{a_1} s_{a_1} S_{a_2} s_{a_2}	Complementary factors for the stigma color S_{a_1} is linked with g_1—crossover = 18.51 percent Independent of p_{r_1}, p_{r_2}, g_1, g_2 and s_p s_{a_1} may be the same factor as a_{p_4} or closely linked with a_{p_4}	9:7
L_{s_1} l_{s_1} L_{s_2} l_{s_2}	Complementary factors for the leaf-sheath color l_{s_1} is linked with g_1—crossover = 19.43 percent They are independent of p_{r_1}, p_{r_2}, g_1, g_2 and s_p l_{s_2} is closely linked with s_{a_2}—crossing over 9.8 percent	9:7

FACTORS	CHARACTERS FOR WHICH THE FACTORS ARE RESPONSIBLE	RATIOS
L_{s3} l_{s3} L_{s4} l_{s4}	Duplicate factors for the leaf-sheath color	15:1
L_{g1} l_{g1} L_{g2} l_{g2} L_{g3} l_{g3}	Complementary factors for the ligule color They are independent of g_1, g_1, g_2 and s_p l_{g3} may be the same factor as p_{r2} or completely linked with it	27:37
H_1 h_1 H_2 h_2	Complementary factors for the black hull Independent of l_{s3}, l_{s4}, a_{p6}, p_{r1} and p_{r2}	9:7
I_{n1} i_{n1} I_{n2} i_{n2}	Complementary factors for the internode color They are independent of h_1 and h_2	9:7

While the results outlined above are self-explanatory, a few of them may be briefly discussed with advantage. First of all, it is interesting to note that the awn, being a sporophytic character, should be either present or absent on all the spikelets of the same plant in the F_2 generation. Contrary to this expectation, two new types have appeared. One type has most of the spikelets awned, and the other has only a few spikelets awned, the remaining spikelets of the same panicle being awnless, as shown in figure 1. These new types and the original grand-parental types occur in about a 12:1:2:1 ratio. The observed frequencies have been explained on the basis of two duplicate factors. The awn may be conceived of as an extension of the central nerve of the lemma. The gene, A_{n1}, extends the central nerve either in single or double dose with or without A_{n2}. The gene A_{n2} is of similar nature, but it is weaker in activity; so, the double doses may extend most of the central nerves, while the single dose may extend just a few of them. Environmental conditions may also enter in, thus influencing the action of A_{n2} during the morphogenesis of the spikelets and particularly of the awn. As the spikelets on the top of the panicle and those on the lower part do not develop at the same time, we can easily see the differential action of the gene under different conditions in the extension of the awn. It is interesting to note that in the "mostly awned" and "rarely awned" classes, usually it is the spikelets at the lower part of the panicle whose awns are not extended.

Factorially, the case may be represented as follows:

$$\left.\begin{array}{l} 1\ A_{n1}\ A_{n1}\ A_{n2}\ A_{n2} \\ 2\ A_{n1}\ A_{n1}\ A_{n2}\ a_{n2} \\ 2\ A_{n1}\ a_{n1}\ A_{n2}\ A_{n2} \\ 4\ A_{n1}\ a_{n1}\ A_{n2}\ a_{n2} \\ 1\ A_{n1}\ A_{n1}\ a_{n2}\ a_{n2} \\ 2\ A_{n1}\ a_{n1}\ a_{n2}\ a_{n2} \end{array}\right\} = 12 \text{ fully awned}$$

1 a_{n_1} a_{n_1} A_{n_2} A_{n_2}=1 mostly awned
2 a_{n_1} a_{n_1} A_{n_2} a_{n_2}=2 rarely awned
1 a_{n_1} a_{n_1} a_{n_2} a_{n_2}=1 wholly awnless

Several factors have been shown to lie on the glutinous chromosome. T_y is coupled with G_1, giving 16.59 percent crossing over. a_{p_4} is linked with g_1, giving 22.34 percent crossing over. l_{s_1} is also coupled with g_1, giving 19.43 percent crossing over. These constitute the first linkage group which may be expressed in the following diagram:

$$a_{p_4} \quad l_{s_1} \quad t_y(?) \quad g_1 \quad t_y(?)$$

Whether t_y is on the left or right hand side of g_1 is not known at present. It may be noted that s_{a_1} is also linked with g_1, giving about 18.51 percent crossing over. However, whether s_{a_1} is the same factor as l_{s_1}, or a separate gene closely linked with l_{s_1} remains to be determined. It may be further noted that TAKAHASHI's factor (R) for awn color and YAMAGUCHI's factor (S) for apiculus color are also in the same linkage group, though their exact loci cannot be stated. It is possible that (S) is the same factor as s_{p_4}.

The second linkage group constitutes two factors, namely, s_p and g_2. The glume length depends on two duplicate factors, g_1 and g_2, segregating in a 15:1 ratio long glume being recessive. The spikelet length depends on a simple factor pair, S_p s_p, long spikelet being recessive. The factors g_2 and s_p are coupled, giving 1.11 percent crossing over. That the short spikelet factor, S_p, is coupled with one of the duplicate glume genes (G_2) is beyond doubt. It is of interest to note, however, that on the basis of 1.11 percent crossing over, only 0.99 or one plant in a population of 718 individuals is expected to have short spikelets and long glumes, and one such individual has been obtained as shown in figure 3 (F_2 SL). That this individual is a crossover is proved by the fact that there is no such variety in my stock that has short spikelets and long glumes, thus eliminating any error through contamination. It may further be pointed out that if the observed crossover plant had not appeared, the data (see table 10) could be explained equally well on the basis of 12:0:3:1 ratio by assuming that one of the duplicate glume factors is the same as the S_p factor for the spikelet length. The difficulty, however, is that on this assumption, it must follow that the long spikelet plant must necessarily have long glumes also. But this is not the case.

The third linkage group consists of s_{a_2} and l_{s_2}. The purple stigma in this case depends upon the presence of two dominant complementary factors, namely, S_{a_1} and S_{a_2}. The purple leaf-sheath also depends on two comple-

mentary factors, L_{s_1} and L_{s_2}. Since both L_{s_1} and S_{a_1} are linked with G_l, giving about the same percentage of crossing over, L_{s_1} and S_{a_1} may be the same factor. For the sake of convenience, a common factor (A) is assumed to represent both L_{s_1} and S_{a_1}, though the assumption must await further verification. It is certain, however, that linkage does exist between the characters, no matter what assumption we may make. The observed frequencies cannot be explained on the independent segregation of either four or three factors, as shown in table 22a. It appears that only on the assumption of a close linkage between S_{a_2} and L_{s_2} can we explain the observed frequencies. In so doing, the crossing over value is found to be 9.8 percent between s_{a_2} and l_{s_2}. The calculated zygotic ratios on this basis fit the observed frequencies closely.

Thus far, three linkage groups have been established beyond doubt. A fourth group is indicated by the data presented in table 25. The observed frequencies can be explained by two alternatives. One is that one of the pericarp factors is the same as one of the three ligule factors, thus giving a ratio of 108:0:132:16 on the basis of four factors, that is the two characters have one factor in common. The other alternative is that one of the pericarp factors is completely or very closely linked with one of the three ligule factors. The occurrence of one individual having "purple ligule and colorless pericarp," which cannot be accounted for by the first alternative, favors the second view. In this connection, the writer takes the liberty to rearrange HECTOR'S (1922) data in the following table for comparison with his own results:

	PURPLE LIGULE COLORED PERICARP	PURPLE LIGULE COLORLESS PERICARP	COLORLESS LIGULE COLORED PERICARP	COLORLESS LIGULE COLORLESS PERICARP	TOTAL
Observed (Hector)	1220	6	951	730	2907
Observed (Chao)	107	1	131	16	255

It must be noted, however, that though the two sets of observed frequencies are similar in nature, they are not exactly comparable. For in HECTOR'S case, the color of the pericarp depends on one factor pair segregating in a 3:1 ratio, while in the present case, the pericarp color is due to duplicate factors segregating in a 15:1 ratio. The common feature in both cases is that on the assumption of a common factor for both pericarp and ligule color, these few exceptions having "purple ligule and colorless pericarp" can not be accounted for. On the other hand, these exceptional individuals tend to support the view that one of the ligule

factors is closely linked with one of the factors responsible for the pericarp color.

Finally, it is interesting to note that so many of the characters studied are due to duplicate genes. This is of particular interest in view of the fact that all the varieties used have twenty-four chromosomes.

SUMMARY

1. Data have been presented to show the Mendelian behavior and chromosomal relations of twenty-five or more genetic factors which affect one or another part of rice plants.

2. Of the twelve characters studied, five are due to duplicate genes, each segregating in a ratio of 15:1.

3. Through the study of the interrelations between these twenty-five genes, three linkage groups have been established beyond doubt and possibly a fourth group is also indicated.

4. The first linkage group consists of four or five factors, namely, g_l, a_{p_4}, l_{s_1}, t_y, and possibly s_{a_1}.

5. There is 16.59 percent crossing over between g_l and t_y; 18.51 percent between g_l and s_{a_1}, 19.43 percent between g_l and l_{s_1}, and 22.34 percent between g_l and a_{p_4}. In addition a_{p4} is very closely linked with l_{s_1}.

6. The second linkage group consists of two genes, namely, s_p and g_2. The glume length depends on duplicate factors, g_1 and g_2, segregating in a 15:1 ratio, long glume being recessive. The spikelet length depends on a simple factor pair, S_p s_p, long spikelet being recessive. One of the duplicate genes, presumably g_2, is coupled with sp, giving 1.11 percent crossing over.

7. The third linkage group consists of s_{a_2} and l_{s_2}. Purple stigma in this case depends upon the presence of two dominant complementary factors, namely, S_{a_1} and S_{a_2}. Purple leaf-sheath also depends on two complementary factors, L_{s_1} and L_{s_2}. Between these two characters, there is close linkage. Only on the assumption of coupling between S_{a_2} and L_{s_2} can the observed frequencies be explained. BRUNSON'S method for calculating linkage intensities involving complementary factors is further modified for the present case. The crossing over value is found to be 9.8 percent between s_{a_2} and l_{s_2}.

8. A fourth linkage group is indicated between p_{r1} or p_{r2} and one of the three complementary factors for purple ligule color.

9. Factors g_1 and g_2 are independent of g_l, p_{r_1}, and p_{r_2}.

10. The factor s_p is independent of g_l, p_{r_1}, p_{r_2}.

11. Factors a_{p_1} and a_{p_2} are independent of g_l.
12. Gene a_{p_4} is independent of p_{r_1}, p_{r_2}, g_1, g_2, and s_p.
13. Factors s_{a_1} and s_{a_2} are independent of p_{r_1}, p_{r_2}, g_1, g_2, and s_p.
14. Factors l_{s_1} and l_{s_2} are independent of p_{r_1}, p_{r_2}, g_1, g_2, and s_p.
15. Factors l_{q_1}, l_{q_2}, and l_{q_3} are independent of g_l, g_1, g_2, and s_p.
16. Factors h_1 and h_2 are independent of i_{n_1}, i_{n_2}, l_{s_3}, l_{s_4}, a_{p_5} a_{p_6}, p_{r_1}, and p_{r_2}.

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APPENDIX—TABLES 29–45

TABLE 29

Independent segregation between G_1, G_2 and G_1 from the cross (4269×4957).

PHENOTYPES	OBSERVED	*Calculated 45:3:15:1*	DEVIATION	$\frac{(O-C)^2}{C}$
Non-glutinous short glume	525	*504*	21	0.87
Non-glutinous long glume	34	*33.6*	0.4	0.05
Glutinous short glume	146	*168*	−26	2.88
Glutinous long glume	12	*11.2*	0.8	0.05
Total	717	*717*		$3.85 = X^2$

$P = 0.2810$

TABLE 30

Independent segregation between G_1, G_2 and P_{r_1}, P_{r_2} from the cross (4269×4957).

	SHORT GLUME COLORED PERICARP	SHORT GLUME COLORLESS PERICARP	LONG GLUME COLORED PERICARP	LONG GLUME COLORLESS PERICARP	TOTAL
Observed	590	46	42	1	679
Calculated 225:15:15:1	*596.25*	*39.75*	*39.75*	*2.85*	*679*
	−6.25	6.25	2.25	−1.65	

$P = 0.495$

TABLE 31

Independent segregation between $S_p s_p$ and $G_{1}g_{1}$ in F_2.

	SHORT SPIKELET NON-GLUTINOUS	SHORT SPIKELET GLUTINOUS	LONG SPIKELET NON-GLUTINOUS	LONG SPIKELET GLUTINOUS	TOTAL
Observed	417	118	138	40	713
Calculated 9:3:3:1	*400.5*	*133.5*	*133.5*	*44.5*	*713*
Deviation	15.5	−15.5	4.5	−4.5	

$X^2=4.34$. $P=0.2309$.

The deviation is apparently due to the large deficiency of glutinous grains.

TABLE 32

Independent segregation between factors s_p, p_{r_1}, and p_{r_2} (Ratio 45:4:15:1).

PHENOTYPES	OBSERVED	*Calculated*	$\frac{(O-C)^2}{C}$
Short spikelet, colored pericarp	505	*501.75*	0.02
Short, colorless	32	*33.45*	0.06
Long, colored	169	*167.25*	0.01
Long, colorless	8	*11.15*	0.89
	714	*714.0*	$0.98=X^2$

When $X^2=1$, $P=0.8013$.

TABLE 33

Showing independent segregation between A_{p_4} and p_{r_1} and p_{r_2}.

	COLORED APEX COLORED PERICARP	COLORLESS APEX COLORED PERICARP	COLORED APEX COLORLESS PERICARP	COLORLESS APEX COLORLESS PERICARP	TOTAL
Observed	500	162	38	9	709
Calculated 45:15:3:1	*498.15*	*166.05*	*33.21*	*11.07*	*709*
Deviation	1.85	−4.05	5.21	−2.07	

$X^2=0.982$. $P=0.801253$ when $X^2=1$.

TABLE 34

Showing independent segregation between a_{p_4} and g_1 and g_2.

	COLORED APEX SHORT GLUMES	COLORLESS APEX SHORT GLUMES	COLORED APEX LONG GLUMES	COLORLESS APEX LONG GLUMES	TOTAL
Observed	525	146	25	16	712
Calculated 45:15:3:1	*500.40*	*166.80*	*33.36*	*11.12*	*712*
Deviation	24.55	−20.80	−8.36	4.88	

$X^2=7.839$. $P=0.0418$.

The cause of the deviation is unknown.

TABLE 35

Showing independent segregation between A_{p_4} and s_p.

	COLORED APEX SHORT SPIKELET	COLORLESS APEX SHORT SPIKELET	COLORED APEX LONG SPIKELET	COLORLESS APEX LONG SPIKELET	TOTAL
Observed	428	133	139	43	743
Calculated 9:3:3:1	*417.87*	*−139.29*	*139.29*	*46.43*	
Deviation	10.13	−6.29	−0.29	−3.43	

$X^2=0.788$ $P=0.801253$ when $X^2=1$.

TABLE 36

Showing independent segregation between s_{a_1}, s_{a_2} and p_{r_1}, p_{r_2}.

	PURPLE STIGMA COLORED PERICARP	COLORLESS STIGMA, COLORED PERICARP	PURPLE STIGMA COLORLESS PERICARP	COLORLESS STIGMA, COLORLESS PERICARP	TOTAL
Observed	204	158	27	8	397
Calculated 135:105:9:7	*209.25*	*162.75*	*13.95*	*10.85*	*397*
Deviation	−5.25	−4.75	13.05	−2.85	

The deviation is probably due to the small number of plants.

TABLE 37

Showing independent segregation between s_{a_1}, s_{a_2} and g_1, g_2.

	PURPLE STIGMA COLORED PERICARP	COLORLESS STIGMA, COLORED PERICARP	PURPLE STIGMA COLORLESS PERICARP	COLORLESS STIGMA, COLORLESS PERICARP	TOTAL
Observed	227	163	12	16	418
Calculated 135:105:9:7	*220.32*	*171.36*	*14.68*	*11.42*	*418*
Deviation	6.68	−8.36	−2.68	4.58	

$X^2=2.92$. $P=0.405996$.

TABLE 38

Showing independent segregation between s_{a_1}, s_{a_2} and s_p.

	PURPLE STIGMA SHORT SPIKELET	COLORLESS STIGMA, SHORT SPIKELET	PURPLE STIGMA LONG SPIKELET	COLORLESS STIGMA, LONG SPIKELET	TOTAL
Observed	189	130	53	49	421
Calculated 27:21:9:7	*177.39*	*137.97*	*59.13*	*45.99*	*421*
Deviation	11.61	−7.97	−5.13	3.01	

$X=1.85$. $P=0.6067$.

TABLE 39

Showing independent segregation between L_{s_1}, l_{s_2} and p_{r_1}, p_{r_2} (4269×4957).

	COLORED PERICARP COLORED SHEATH	COLORED PERICARP COLORLESS SHEATH	COLORLESS PERICARP COLORED SHEATH	COLORLESS PERICARP COLORLESS SHEATH	TOTAL
Observed	179	134	19	4	336
Calculated 135:105:9:7	*177.12*	*137.76*	*11.80*	*9.18*	*336*
Deviation	1.88	−3.76	7.20	−5.18	

The deviation is probably due to the small number of plants.

TABLE 40

Showing independent segregation for l_{s_1}, l_{s_2}, g_1 and g_2.

	COLORED LEAF SHEATH SHORT GLUMES	COLORLESS SHEATH SHORT GLUMES	COLORED SHEATH LONG GLUMES	COLORLESS SHEATH LONG GLUMES	TOTAL
Observed	192	128	11	11	342
Calculated 135:105:9:7	*180.22*	*140.17*	*12.01*	*9.35*	*342*
Deviation	11.78	−12.17	−1.01	1.65	

$X^2=2.7$. $P=0.4458$.

TABLE 41

Showing independent segregation for l_{s_1}, l_{s_2} and s_p.

	COLORED LEAF SHEATH SHORT SPIKELET	COLORLESS SHEATH, SHORT SPIKELET	COLORED SHEATH LONG SPIKELET	COLORLESS SHEATH, LONG SPIKELET	TOTAL
Observed	154	100	50	39	343
Calculated 27:21:9:7	*144.72*	*112.56*	*48.24*	*37.52*	*343*
Deviation	9.28	−12.56	1.76	1.48	

$X^2=2.1$. $P=0.5543$.

TABLE 42

Showing independent segregation of g_1, g_2, l_{a_1}, l_{a_2} and l_{a_3}.

	PURPLE LIGULE SHORT GLUMES	PURPLE LIGULE LONG GLUMES	GREEN LIGULE SHORT GLUMES	GREEN LIGULE LONG GLUMES	TOTAL
Observed	104	5	134	13	256
Calculated (5 factors)	*101.25*	*6.75*	*138.75*	*9.25*	*256*
Deviation	2.75	−1.75	−4.75	3.75	

Ratio = 405:27:555:37

TABLE 43

Showing independent segregation between l_{g_1}, l_{g_2}, l_{g_3} and s_p.

	PURPLE LIGULE SHORT SPIKELET	PURPLE LIGULE LONG SPIKELET	GREEN LIGULE SHORT SPIKELET	GREEN LIGULE LONG SPIKELET	TOTAL
Observed	87	21	108	40	256
Calculated 81:27:111:37	*81*	*27*	*111*	*37*	*256*
Deviation	6	−6	−3	3	

$X^2=2.09$, $P=0.5561$.

TABLE 44

Showing independent segregation between h_1, h_2, a_{p_5} and a_{p_6}.

	BLACK HULL COLORED APICULUS	NON-BLACK COLORED APEX	BLACK HULL COLORLESS APEX	NON-BLACK COLORLESS APEX	TOTAL
Observed	109	92	5	12	218
Calculated 135:105:9:7	*114.75*	*89.25*	*7.65*	*5.95*	*218*
Deviation	−5.75	2.75	−2.65	6.05	

The deviation is probably due to the small number of plants.

TABLE 45

Showing independent segregation between h_1, h_2, p_{r_1} and p_{r_2}.

	BLACK HULL COLORED PERICARP	NON-BLACK COLORED PERICARP	BLACK HULL COLORLESS PERICARP	NON-BLACK COLORLESS PERICARP	TOTAL
Observed	106	89	7	13	215
Calculated 135:105:9:7	*113.4*	*88.2*	*7.56*	*5.88*	*215*
Deviation	−7.4	0.8	−0.56	7.12	

The deviation is probably due to the small number of plants.

LINKAGE OF THE FACTORS FOR SHORT-EAR AND DENSITY IN THE HOUSE MOUSE (*MUS MUSCULUS*)

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INTRODUCTION

In 1921 Miss LYNCH reported a mutation in the house mouse in the form of "short-ears." The ears of this mutant are short, thick, and somewhat wrinkled. Correlated with this are modifications of the skull such as shortening of the muzzle, curvature of the frontal bones, and other structural variations.

Miss LYNCH reported this as a simple recessive mutation, the gene for which was located in an autosome. The author's observations are in harmony with Miss Lynch's, for he has obtained 397 short-eared individuals out of an F_2 population of 1613, where expectation is 403, a difference of only 6 ± 11.7.

This short-eared character, from a morphological standpoint, seems similar to that described by RITZMAN (1916) in sheep. In both cases the ears are short and thick, and both are due to a single Mendelizing factor. The two characters differ, however, in their inheritance. Short-ears in sheep is dominant, while in mice it is recessive.

In February, 1925, Miss Lynch very kindly sent to Doctor W. E. CASTLE of HARVARD UNIVERSITY six short-eared males. These were mated to normal mice, mostly pink-eyed, dilute browns. The F_2 from this out-cross gave a number of short-eared individuals, from which a strain of short-eared mice was established. These were inbred for three generations and proved constant for the short-ear character.

The author wishes to thank Doctor CASTLE for the gift of these short-eared mice, which constituted the original source of his stock. He also wishes to express his appreciation of kindly suggestions of this report by Doctor CASTLE.

During the winter and spring of 1925, the author, GATES (1926), tested this short-ear mutation as to linkage relationship with the waltzing factor. It showed itself to be inherited independently of this character. KEELER (1926) found it to be independent of the factor for rodless retina. Further than this, the author is not aware that the short-eared character has been tested for linkage with any other character aside from that reported herewith.

Since the most satisfactory method of testing linkage is a back-cross to a double recessive, and since the author had a strain of pink-eyed, dilute, brown, piebald waltzers, the short-ear was crossed to this strain in the hope of establishing a strain of pink-eyed, dilute, brown, piebald, short-eared waltzers, and then crossing these with the dense black, short-eared strain, in this manner testing the short-eared character for linkage with pink-eye, dilution, brown, piebald, and waltzing at one and the same time.

It was very quickly evident, however, that there was linkage between short-ear and density, for from the first, the dilute, short-eared class was lacking, although the other recombination classes showed up as expected.

With linkage between short-ear and density apparent, the cross involving the five point recessive was abandoned so as to eliminate the unequal growth conditions in litters containing both waltzers and non-waltzers. The parent races then selected were Doctor LITTLE'S strain of dilute browns, and a pink-eyed, dense, short-eared strain. I am indebted to Doctor LITTLE for kindly sending me several pens of dilute mice which were mated with the short-eared race.

The F_1 from this cross proved to be quite vigorous and hardy. The females were good mothers, seldom killing the young as was sometimes the case in the cross which involved several recessive characters including waltzing.

The character dilution involved in this experiment is that described and analyzed by Miss DURHAM (1908, 1911), LITTLE (1913), and others. It is that factor which dilutes the black color of mice to a maltese gray; and changes the chocolate brown color to a taupe. As summarized by the author, GATES (1926), this dilute factor has been shown to be inherited independently of the factors for agouti, brown, pink-eye, piebald, and waltzing. WACHTER (1927) reports it to be independent of black-eyed white.

METHODS

The mice were kept in wooden boxes with wire tops. Breeding pens were made up of not over four and usually only three females mated to

one male. Except in small litters, where there was no male, all mice of each generation were brother-sister mated.

Pregnant females were removed from the breeding pens and placed in separate boxes. The young were recorded at birth and the female left undisturbed for fourteen days. On the fourteenth day or after, the young were examined and their characters recorded.

While the characters of dilution and density are very early distinguishable (fourth to sixth day), those of short- and normal-ear cannot be determined before the young are from fourteen to sixteen days old. Occasionally, retarded growth may not enable one to distinguish the characters even at that age.

At three weeks or later, the young were weaned and their recorded characters checked. The mother was then returned to her pen. and the young mated or discarded.

All F_2 young which resembled their F_1 parents with respect to the two characters were mated *inter se* or with others like themselves. The short-eared individuals (which were all dense) were mated with the dilute and the dilute individuals with short-eared. The symbols used herewith are those adopted at New Haven, 1925, GATES (1926), namely, density—*D*, dilution—*d*, normal-ear—S^e, and short-ear—s^e.

RESULTS

When two independent Mendelian characters enter a cross they tend to segregate in the F_2 in the ratio of 9 showing both dominant characters, 3 showing one dominant and one recessive, 3 showing the other dominant and other recessive, and 1 showing both the recessive characters. Linkage will tend to disturb this ratio, and if it is quite strong, with crossing over very rare, it is possible that two cross over gametes will hardly ever unite, forming the double recessive. Complete linkage will eliminate the last class entirely and give a ratio in the other three classes of 2:1:1, in which 2 will show both dominant characters, 1 one of the recessives, and 1 the other recessive.

In this particular case the hybrid between a short-eared dense mouse and a normal-eared dilute is normal-eared and dense, as would be expected. When mated together these hybrids, if there is no linkage between short-ear and dilution, should theoretically produce four types of offspring, namely, normal-eared dense, normal-eared dilute, short-eared dense, and short-eared dilute in the ratio of 9:3:3:1.

Actually, however, these F_1 double heterozygotes produced only three types, namely, normal-eared dense, short-eared dense, and normal-eared

dilute. These have, up to this time, occurred in the ratio of 504:234:236. Theoretically, with complete linkage, the expected ratio in a population of this size would be 487:243.5:243.5, a difference of 17 ± 10.53, 9.5 ± 9.11, and 7.5 ± 9.11 respectively.

The probable error of the first class is computed on the basis of a 1:1 ratio and the other two on a 3:1 ratio of the total population. Obviously, therefore, the deviation is so slight as to be well within the range of probability of a 2:1:1 ratio. It is to be noted, however, that the deviations from a 2:1:1 ratio are all *qualitatively* such as would be expected if linkage were *not quite* complete, that is if a small amount of crossing over actually occurred. Thus the first class is greater than expected and the other two classes are both less than expected. However, the variations in the numbers from time to time during the experiment would indicate that the deviations were due to random sampling.

The entire absence of the fourth class indicates very close or possibly complete linkage. It indicates that if crossing over occurs at all, it is extremely rare. It suggests as a bare possibility that crossing over may in this instance be restricted to one sex, as in Drosophila. But this suggestion, aside from facts reported below, is rendered highly improbable by the repeatedly verified fact that in mice crossing over occurs almost as freely in males as in females between the genes *c* (albinism) and *p* (pinkeye,), (CASTLE 1925). The situation is similar as regards crossing over in rats, (CASTLE and WACHTER 1924), while in rabbits no significant difference is found between the sexes as regards frequency of crossing over, (CASTLE 1926).

A short-eared dilute individual would result only from a double cross over, that is, a cross over in both the gametes which united to form the zygote. The numbers are sufficiently large to make it obvious, therefore, that if crossing over is not restricted to one sex, it is so rare that there is little or no probability of two crossover gametes uniting.

From the results recorded above there is nothing to indicate that a crossover might not have taken place in one of the two gametes producing a zygote. In other words, the three phenotypes mentioned above might each possess a genetic constitution of a crossover in one of the two gametes, without altering its appearance. Thus the normal-eared dense individuals of the F_2 generation might have a genetic formula of S^eS^eDd, S^eS^eDD, S^eS^eDd, or S^eS^eDD. A genetic constitution indicated by either the second or the third formula would mean that a crossover had taken place in one of the two constituent gametes but not in the other, while a genetic

constitution indicated by the last formula could result only from the union of two crossover gametes.

The genetic formula of each of these normal-eared dense individuals of the F_2 generation could be determined by mating to similar sibs and noting the F_3 generation, or by mating first to a short-eared and then to a dilute individual. The former mating being simpler, and usually involving less time, and fewer offspring, was the type of mating used to test the normal-eared dense individuals of the F_2. Such matings might be of ten possible conbinations, namely:

$$\begin{aligned}
&S^eS^eDd \times S^eS^eDd\\
&\quad\text{“}\quad \times S^eS^eDd\\
&\quad\text{“}\quad \times S^eS^eDD\\
&\quad\text{“}\quad \times S^eS^eDD\\
&S^eS^eDd \times S^eS^eDd\\
&\quad\text{“}\quad \times S^eS^eDD\\
&\quad\text{“}\quad \times S^eS^eDD\\
&S^eS^eDD \times S^eS^eDD\\
&\quad\text{“}\quad \times S^eS^eDD\\
&S^eS^eDD \times S^eS^eDD
\end{aligned}$$

A mating of the first combination, that is of double heterozygotes similar to the F_1 generation, would alone produce the three types of individuals, normal-eared dense, short-eared dense, and normal-eared dilute. Any of the nine other possible matings would produce only one, or at most two classes of young, but never three. The normal-eared dense individuals of the F_2 generation can thus, by mating *inter se*, be tested for crossovers in one or the other of the gametes which produced them.

Such matings have given the following total numbers of individuals: 177 normal-eared dense, 90 short-eared dense, and 94 normal-eared dilute. These results are so close to the 2:1:1 expectation that there is small probability of a crossover having occurred, even if these individuals had not been further tested, for on the basis of a 2:1:1 ratio, the expectation is 180.5:90.25:90.25, a difference of 3.5 ± 6.41, 0.25 ± 5.55, 3.75 ± 5.55. Here it is to be noted that qualitatively the deviations are the reverse as would occur if crossing over were an occasional or rare event, for the double dominant class is *less* than expected and the single dominant class is *greater* than expected.

Furthermore, 54 males and 66 females of normal-eared dense animals of the F_2 generation have been tested *individually* and have produced all three classes of young, showing themselves to have a genetic constitution similar to their parents of the F_1 generation. In every instance, where there have been a sufficient number of young, the parents have proved to be double heterozygotes by producing the three types of young, indicating a condition of no crossing over.

Similarly, the normal-eared dense individuals of the F_3 and F_4 generations were tested by mating *inter se*. The results of these matings show a ratio of 146:69:74, the expectations (if linkage is complete) being 144.5:72.25:72.25 respectively, and the deviations from these expectations being 1.5 ± 5.73, 3.25 ± 4.97, and 1.75 ± 4.97 respectively. Here again the results follow closely the expectation of 2:1:1 and give no indication either qualitative or quantitative of crossing over. The matings in question have been made between 35 males and 41 females of the F_3 and F_4 generations and each mating has yielded in every instance the expected three classes of young.

Since there is little or no indication from the above results that a crossover has occurred or that the normal-eared dense individuals are anything but double heterozygotes, we may combine these figures to obtain a total progeny population of 1624 for matings *inter se* of double heterozygotes, which will include 827 normal-eared dense, 393 short-eared dense and 404 normal-eared dilute, the respective expectancies being 812:406:406. Here the deviations from expectancy on the basis of 2:1:1 ratio are 15 ± 13.5, 13 ± 11.7, and 2 ± 11.7. This is a very close approximation to expectancy and tends toward the same possible qualitative result shown by the F_2 generation. But here again the results have varied from time to time and are not at all consistent. For instance, at an earlier stage in the experiment the numbers were 735:360:370 with expectation of 732.5:366.25:366.25, a deviation of 2.5 ± 12.8, 6.25 ± 11.0, and 3.75 ± 11.0 This can hardly indicate anything but random sampling.

Futhermore, combining the normal-eared dense individuals of the F_2, F_3, and F_4 generations, which have been individually tested, we find a total of 224, both males and females. In no instance where the number of offspring has been sufficient has any individual of this number failed to produce all three types of young, showing that no crossing over has occured in any of these double heterozygotes, and an indication that if crossing over occurs at all, it is a very rare event.

Each of the other two classes of the F_2 generation might just as well be the result of the union of one crossover gamete with a non-crossover.

Under these conditions the short-eared dense individuals would have a formula of $s^e s^e Dd$ and the normal-eared dilute $S^e s^e dd$, whereas the non-crossover formulae would be $s^e s^e DD$ and $S^e S^e dd$ respectively.

Both of these classes can be simultaneously tested for a crossover gamete by mating them with each other: short-eared dense with normal-eared dilute. Such matings, on the hypothesis of complete linkage, should produce only one type of young, normal-eared dense, similar in constitution to the F_1. If there should appear among the offspring a recessive individual, either short-eared or dilute, one or the other parent must have been produced by a gamete carrying a crossover. Thus, $s^e s^e Dd$ (crossover) $\times S^e S^e dd = S^e s^e Dd$ and $S^e s^e dd$ (dilute) or $S^e s^e dd$ (crossover) $\times s^e s^e DD = S^e s^e Dd$ and $s^e s^e Dd$ (short-eared). Futhermore, if this mating should produce three or four classes of young, it would indicate a crossover in one gamete of each parent, for $S^e s^e dd \times s^e s^e Dd = S^e s^e Dd + S^e s^e dd + s^e s^e Dd + s^e s^e dd$.

Up to date, 58 short-eared males and 66 short-eared females of the second, third, and fourth generations have been so tested, and in no case have the offspring been anything but normal-eared and dense. In some few instances the number of young has not been large enough to preclude the possibility of the missing class appearing among a larger population. But since expectation is half and half of each of the two possible classes in each case of a single crossover, the author has considered five offspring to constitute an adequate test. Likewise, 32 dilute males and 46 dilute females have been tested and all have proved to be homozygous for normal-ear as well as dilution. None has shown itself to have been produced by a crossover gamete.

Thus we find that both the short-eared and the dilute individuals of the F_2, F_3, and F_4 generations are homozygous for each of the two characters respectively, and therefore are like the original parental types.

Combining all these numbers we have a total of 426 individuals whose genetic constitution has been tested. Since each zygote is the product of two gametes, we may therefore conclude that 852 gametes have been tested with no case of a crossover, indicating a condition of very close or a possibility of complete linkage. Likewise, the further data afforded by the F_2, F_3, and F_4 generations tends to indicate rare or no crossing over.

Under such abnormal Mendelian ratios one must consider the possibility of lethal effects of a homozygous double recessive, therefore, at first, considerable attention was given to checking up all post-natal deaths. It was soon apparent, however, that there were no post-natal

lethal effects, for among the undepleted litters the same ratio was being obtained as among the general population. Less attention was then given to depletion of litters and the figures include both depleted and undepleted litters.

In Drosophila, MORGAN (1914) has shown that no crossing over occurs in the male. CASTLE (1925) has shown that in rats and mice crossing over is but very slightly greater in females than in males. This possibility was kept in mind in this case by testing both females and males of the F_2 generation. The results showed that all normal-eared dense individuals, both *males* and *females*, were double heterozygotes like their parents. A similar condition was found to be true in the F_3 and F_4 generations. None of those tested proved otherwise.

Likewise, both male and female short-eared individuals of the F_2, F_3, and F_4 generations were tested by mating to dilutes and no crossing over was observed in either sex. The same mating in each case gave a test for a crossover in the opposite sex.

DISCUSSION

This instance of two well established and quite different Mendelian characters showing a relationship of extremely close or complete linkage brings to mind other possible cases of complete linkage, such as the often cited instance of the blue Andalusian fowl, which LIPPINCOTT (1918, 1921) analyzed as complete linkage and not partial dominance.

Reported instances of multiple allelomorphs may prove to be, and can easily be conceived to be cases of complete linkage. Agouti pattern and yellow color have long been considered allelomorphs (Cuenot 1911). But if complete linkage can be shown to exist in other cases, it is possible that agouti and yellow are completely linked characters and not allelomorphs.

Unlike the case reported by GOWEN and GOWEN (1922), there appears to be no factor, dominant or recessive, causing this linkage, for it behaves alike on all outcrosses and there is no instance of crossing over in the F_3, F_4, or F_5 generations, nor is there any indication of crossing over in gametes which go to form heterozygotes.

DANFORTH (1927) has recently called attention to the multiple effects of the gene yellow producing yellow color and obesity. Here again the in erpretation may lie in close linkage of the gene yellow and a gene producing obesity, for the disposition to lay on fat is found in other than yellow mice, while yellow mice will nearly always do so under normal feeding conditions.

These two characters dilute and short-ear are so distinct, each of them having been bred extensively and independently for many years, that it is hardly conceivable that they are allelomorphs or that they are multiple effects of single genes, but, on the contrary, from the data here given, we are drawn to the conclusion that they are produced by two very closely linked factors. Although the number of tested gametes is quite large (852) caution should be observed in stating the degree of linkage. In rats, albinism and red-eye were at one time thought to be completely linked (IBSEN 1920), but later CASTLE and WACHTER (1924) found these to be closely linked with a very small percentage of crossing over. So here, it may be that in a larger population, cases of crossing over may occur. The present data, however, indicates that such cases would occur at least but rarely, and that the production of a short-eared dilute mouse from the present short-eared dense stock will at least involve large numbers.

SUMMARY

The data presented in this paper indicate that there is very close or possibly complete linkage between the gene short-ear and density and their allelomorphs normal-ear and dilution. No crossover has been observed in a single gamete out of a total number of 852. The F_2 generation from this cross includes only three types of individuals, normal-eared dense, short-eared dense, and normal-eared dilute, in the ratio of 2:1:1; the normal-eared dense individuals of the F_2, F_3, and F_4 generations have produced only three phenotypes. The short-eared dense individuals of the F_2, F_3, and F_4 generations when mated with normal-eared dilute sibs produce only the one phenotype—normal-eared dense, showing no crossing over, but homozygosity in every case tested.

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A COMPARISON OF THE CHROMOSOMES OF THE RAT AND MOUSE WITH REFERENCE TO THE QUESTION OF CHROMOSOME HOMOLOGY IN MAMMALS[1]

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The facts presented below owe their general interest to certain theortical considerations which have formed in part the background for a series of mammalian chromosome studies to which the writer has devoted his time for the past seven years. The question with which we are immediately concerned is this: May the chromosomes of the eutherian mammals be considered (in general) as having the same genetic make-up; for example, does the largest chromosome pair of different species carry the same genes or modifications of the same original genes? At first sight this would appear as a highly speculative problem for the cytologist to consider for, obviously, the final answer to this question lies with the geneticists of the future, but if we go on the assumption that the chromosomes represent the material substratum of genetic characters and hence in a sense that they are aggregations of genes, then it is possible for the cytologist to indicate in a provisional way the probable final answer.

The chromosome constitution of animals considered either from the standpoint of number or of size varies within wide limits; nevertheless, it has been a common experience of cytologists working in restricted fields to find that closely related species show a close similarity in their chromosome make-up. There is an easily recognized type number and deviations from this can be explained, usually, on the basis of an end-to-end fusion or to a breaking up (transverse fragmentation) of one or more chromosome pairs. The most striking example of this is found in the Acrididae which McClung and his students have studied so extensively, but animal cytology abounds with illustrations of this same condition as an examination of chromosome tables will convince any one (see HARVEY 1920, or WILSON 1925, p. 855).

The reason why chromosomes of related species are similar is undoubtedly phylogenetic, that is, related species have similar chromosome complexes because they inherited these from a common ancestor. Recent

[1] Contribution No. 212 from the Department of Zoology, UNIVERSITY OF TEXAS, Austin, Texas.

works in genetics and cytology have revealed unsuspected factors which would tend to stabilize this established chromosome constitution and to hold it at a constant level both quantitatively and qualitatively. The localization of sex determining factors in sex chromosomes in animals forms a very effective bar to a change in the chromosomes due to polyploidy as both the writer (PAINTER 1925a, 1925b) and MULLER (1925) have pointed out. There are various conceivable ways in which triploid or even tetraploid individuals could (and probably do) arise in nature, but such individuals could scarcely be perpetuated under natural conditions due to the complications associated with the sex chromosomes.

The loss of a chromosome pair, a whole chromosome or even a part of one chromosome due to what has been termed "genic balance" seems to be barred by the fact that such a loss is accompanied either by low fertility, sterility or low viability. Thus the absence of one of the very small fourth chromosomes in *Drosophila melanogaster* results in individuals of low fertility and viability and nullo-IV flies die. More recently GATES (1927), and the writer (1927) have shown that when only a part of one of the small chromosomes of the mouse is absent, (a normal mate being present) such individuals are extremely frail, and Gates has never been able to produce mice in which both chromosomes were affected presumably because of low viability. In this instance the chromosome involved is one of the smaller elements in a complex of forty chromosomes and represents a very small fraction of the whole chromatin mass. It thus appears that the upsetting of the normal genic balance which accompanies the loss of chromatin would probably bar the further perpetuation of such an aberrant chromosome constitution.

Just what effect the reduplication of a whole chromosome would have upon the individual is not clear (for animals) but the unstable character of the trisomic condition would require very special conditions for its perpetuation in the stable tetrasomic form, requirements which would scarcely obtain in nature.

Since the chromosomes represent directly or indirectly the physical basis of the genes, it follows that these same factors which have operated to keep the chromatin at a fixed level have also operated to stabilize the total number of gene loci and we should expect that nearly related animals having similar chromosome complexes would also show a similarity in the linkage relations of the genes. The work of METZ (1923) on Diptera, the only animal group extensively studied in this regard, seems to justify this expectation.

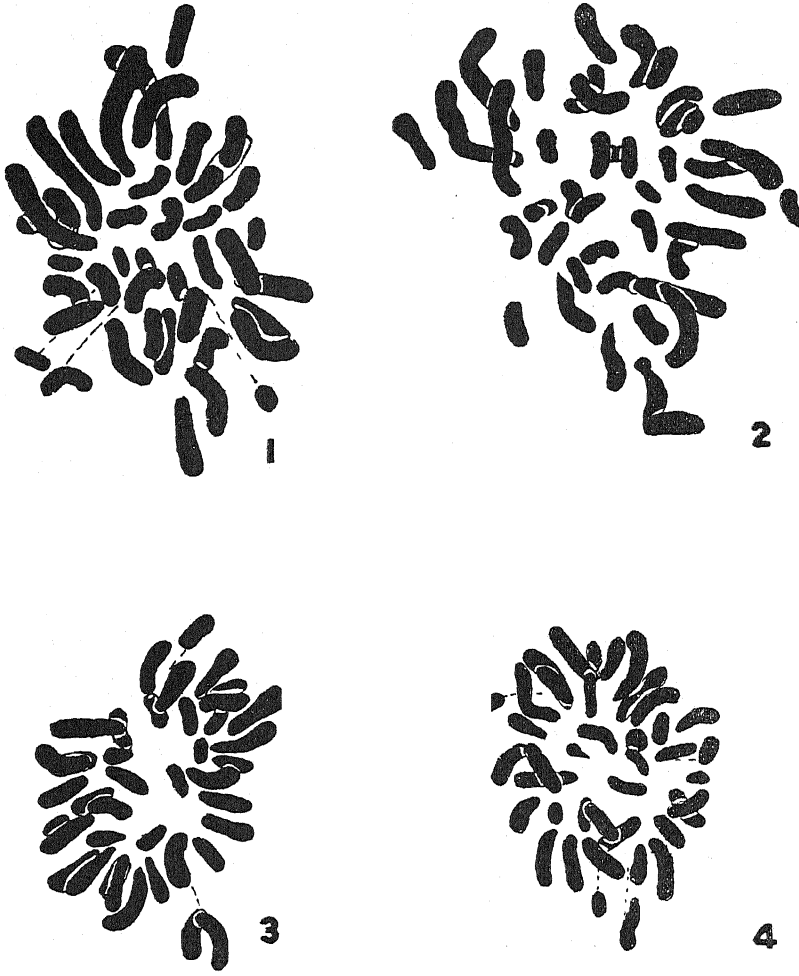
When the writer first began to study the chromosomes of the eutherian

mammals it was anticipated that a great variation in chromosome size and number would be encountered, but as work went forward and species from seven orders were studied it became apparent that the eutheria were much like a genus of insects in this regard. The average and the typical number for the group appears to be about 48, and deviations from this number are accompanied either by an increase in the proportion of small chromosomes, giving a higher number, or by a decrease in the proportion of small chromosomes resulting in a lower count (PAINTER 1925b). These observations accord well with the current conception that deviations from the type number in a small group can be explained on the basis of an end-to-end fusion of two or more pairs, or to a breaking in two of one or more pairs of chromosomes. If these are the only types of changes which have taken place in the eutherian orders and species then there should be an essential homology between the chromosomes of different orders and species and, what is of more interest to geneticists, a similarity in the arrangement of genes except where a break or a fusion has altered linkage values.

On the other hand, as I pointed out in a recent paper (PAINTER 1926), it is possible that, while the chromsome number has been kept at a high level in the eutheria due, perhaps, to some structural peculiarity of the eutherian cells, there may have been a great deal of shifting of chromatin within a chromsome or between non-homologous chromosomes which would greatly affect the arrangement of the genes without altering them in a quantitative way. If this shifting took place within a chromosome such as the inversion of a segment which STURTEVANT and PLUNKETT (1926) have described in *Drosophila simulans* as compared to *D. melanogaster*, it would require careful genetic tests to detect it, but if there has been a considerable shifting of chromatin between non-homologous chromosomes in related species a careful cytological study would reveal it. The extensive study of the chromosomes of rodents which has been under way at this laboratory for several years was undertaken partly with the view of gaining light upon this question.

The white rat (*Mus norvegicus*) and the common house mouse (*Mus musculus*) are placed by systematists in the same genus (*Mus*) and in view of the considerations and observations recorded above, it was anticipated that their chromosome complexes would be very similar. On the contrary, however, the complexes are very different, a fact which forces us to modify the point of view expressed in earlier mammalian chromosome studies.

The material on which this work is based consists of the testes of rats and mice preserved by Allen's modification of Bouin's fluid and subsequently treated in just the same way. In makings drawings much time has been devoted to the careful measurement of the chromosomes, using



FIGURES.—1-4

the reflected image of the camera lucida and a pair of calipers, so that the finished drawing given below are as accurate as it has been possible to make them.

Mus norvegicus possesses 42 chromosomes and typical spermatogonial

chromosomes are shown in figures 1 and 2. These have been measured, copied and placed in serial alinement in figures 5 and 6, due consideration being given in the matching to any foreshortening observed by the microscope. The serial alinements of the elements of figures 1 and 2 (figures 5 and 6 respectively) shows that the rat possesses two pairs of very large



FIGURES.—5–8

chromosomes which are almost double the size of the third largest pair. From the third largest pair downwards the elements form a graded series. The X sex chromosome, as in other eutheria, is a medium sized chromosome but the Y is much larger than in forms heretofore studied. (The evidence for this is based on primary spermatocyte observations). The ratio of size between the smallest and largest elements (computed volume) averages about 1:8.

Mus musculus possesses 40 chromosomes and typical spermatogonial chromosomes are shown in figures 3 and 4. When these are placed in serial alinement (figures 7and 8) the chromosomes form a closely graded series, no element or pair being outstanding in size. The ratio of size between the smallest and largest (computed volume) averages about 1:5.

An attempt to express graphically the differences of the chromosomes of the two species is given in figure 9. The volume of each chromosome has been computed by measuring the length and average thickness as shown by my drawings (volume = length $\times$ thickness^2) and plotting the results in the form of a curve. Curve A (rat) and curve B (mouse)represent averages for the two alinements given for each species. In the rat (A) there is a sharp break in the curve between the second and third chromosome pairs, but from this point downward the seriation is more gradual. In the mouse (B), on the other hand, the seriation of volumes is so gradual that the curve approaches a straight line.

When one compares curves A and B and notes that except for the two large chromosome pairs of the rat, the curves are not unlike, the thought

will suggest itself that either the rat has gained most of the extra chromatin represented by the two largest pairs, or that the mouse has lost this material. It must be remembered, however, that while the unit of measure is the same in both cases (cubic mm for my drawings) that actually the rat spermatogonial cells represented are much greater in size than the

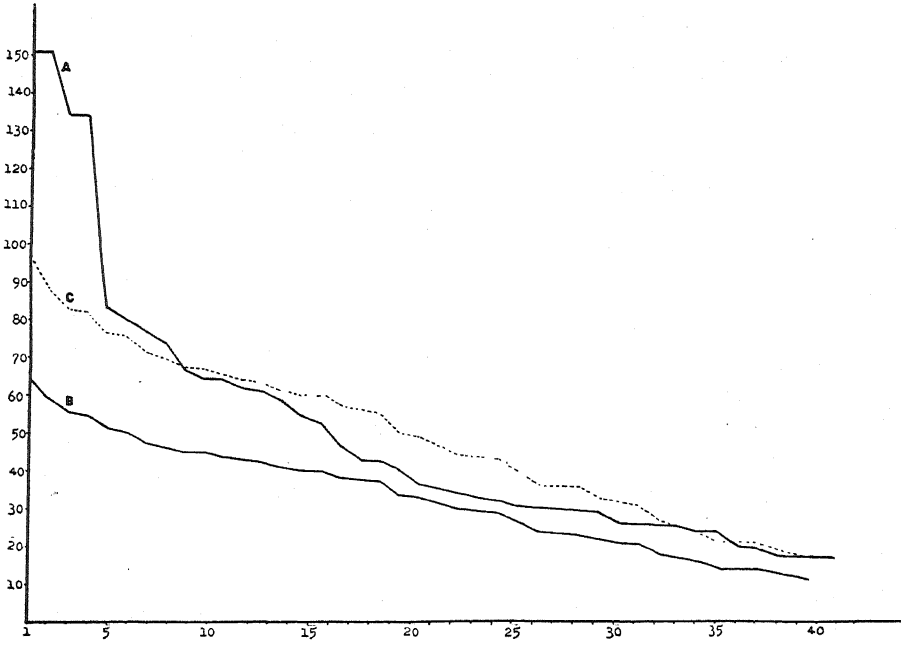


FIGURE 9.—The curves represent the volume of the chromosomes of the rat and mouse, as represented in my drawings, and measured in cubic millimeters. A is *Mus norvegicus*, B, *Mus musculus* and C, is *Mus musculus* with a correction made for difference in cell size between these two species

mouse cells, and since the size of a chromosome varies with the volume of the cell (within limits), we must correct for this difference in volume before we can legitimately draw any conclusions about gain or loss of chromatin.

To gain some idea of the relative volumes of the cells which we are comparing the large and small diameters in each case were measured, averaged and then the volumes computed assuming that the cells were spheres. The ratio of the volumes of the mouse and rat cells illustrated is 28:41. If the volume of the chromosomes, in a given tissue, is proportional to the volume of the cell, we should have to increase the volume of

the mouse chromosomes by about a half in each case to compare them directly with the rat chromosomes. This has been done in C of figure 9. Here we note that while the two large rat chromosome pairs are still far greater in volume than any of the mouse elements, that the volumes of the average sized chromosomes of the mouse are greater than in the rat. The total volume of the rat chromosomes as measured in A is 2130 cubic millimeters. The total volume of the mouse chromosomes (B) is 1327 cubic millimeters. The total volume of the mouse chromosomes as represented by C, is 1990 cubic millimeters. The difference in volume of chromatin in the rat and the mouse, when the difference in cell size is taken into account, is only about 140 cubic millimeters in favor of the rat in spite of the two pairs of large chromosomes and the higher chromosome number.

It would seem, therefore, on the basis of the measurements and calculations given above that the total volume of chromatin in the rat and in the mouse (2130 versus 1990 cubic mm for my drawings) is not very different, when differences of cell size are taken into account, and that we have no good ground for assuming that there has been any extensive loss or gain of chromatin in either species. This conclusion is to be expected, perhaps, in view of the known factors discussed in the first part of this paper which would tend to stabilize the total chromatin content in cells of related animals.

The cells selected for illustrating the chromosomes of the common rat and mouse are typical of what has been found during the past two years at this laboratory. A very large number of cells both somatic and germinal of the two species have come under observation and exact study, and the size relations illustrated have been consistently observed. The method of preservation and the subsequent treatment of the tissues has been the same in both cases. It is conceivable that rat protoplasm and chromatin might react somewhat differently from these structures in the mouse, but the reaction should be uniform, that is, to cause all the chromosomes to swell or shrink proportionally. The differences noted in the alinements of the two species can not be explained on the basis of technique.

The fact that the rat carries the higher number as well as the largest chromosomes precludes the possibility that the observed differences of the two species can be explained on the basis of an end-to-end fusion of two pairs of chromosomes (to give the mouse number) or if the mouse represents the original condition the breaking in two of one pair to give the rat number.

If there has been no great loss or gain of chromatin since the rat and mouse took their origin from a common stem, it is clear that at the present time there is a very different distribution of this material among the chromosomes of the two species. A simple shifting of material between two or three pairs of chromosomes is not adequate to make the alinements similar. If we wish to make the alinement of the rat chromosomes as represented in A of figure 9 similar to that of the mouse (C of figure 9) it would be necessary to remove a large amount of chromatin from the two largest pairs at least and to distribute this to a considerable number of medium sized rat chromosomes, a change which would involve a majority of the rat chromosomes. Or if we wish to make the mouse curve C correspond to the rat alinement, we should have to remove material from a number of medium sized mouse chromosomes and add this to the largest chromosomes. In either case the alterations necessary would be extensive and involve the majority of the chromosomes. We do not know, of course, which of the two species represents more nearly the original stem complex, indeed, the extensive differences which have been noted would suggest that both species have undergone considerable and independent changes in chromatin distribution since they originated.

The fusion of chromosome pairs and the translocation of chromatin have both been reported a number of times for animals but have been considered as comparatively rare phenomena. A comparison of the chromosomes of the rat and mouse, however, seems to indicate that these processes may have played a far more extensive role in vertebrate chromasome organization than has been heretofore thought. Such a shifting of material would not alter the genetic constitution of the animal, and hence would play no direct part in species formation, but it would throw added light upon the problem of the sterility which usually follows species crosses. That this sterility is based upon the failure of the chromosomes to mate up properly in maturation is the generally accepted and doubtless the correct explanation. With the facts just presented concerning the rat and mouse, we can understand better, perhaps, why this is the case. Just because two species look alike and are placed by systematists in the same genus, it does not follow that their chromosomes will be alike. On the contrary they may be very different as in the case immediately before us. This suggests that as a preliminary to species crosses a careful study should be made of the chromosome number and morphology in order to insure as far as this is possible that these are alike before crosses are attempted.

From the cytological standpoint the interpretation which has been given for the differences between the chromosomes of *Mus norvegicus* and *Mus musculus* lends added interest to an intensive study of the chromosomes of nearly related mammalian species in order to determine how frequent and extensive this shifting of material may have been, and how it is accomplished. Both phases are being investigated at this laboratory. At the present time it appears as possible to the writer that this change is accomplished, first, by a breaking up of the chromosomes into a larger number of smaller elements, and subsequently a fusion (without regard perhaps to previous association) of these smaller elements reducing the number again. This possibility is suggested by two sets of observations. In the first place in the wood rat common in the Austin region the chromosome number is high (about 54) and consists of relatively short chromosomes. There are no elements which correspond morphologically to the two largest pairs of chromosomes in *Mus norvegicus*. In the second place it has been observed that in the guinea pig the chromosome number is lower in prophase than in equatorial plate stages which can be interpreted as meaning either that we are witnessing in this form a breaking up of the chromosomes or a fusion of previously fragmented elements. Earlier, I was inclined to the former point of view, but the latter alternative is just as logical and seems now the more probable explanation in view of all the facts.

Since the chromatin furnishes directly or indirectly the physical substratum of the genes we should expect that the extensive redistribution of material which has taken place since the rat and mouse took their origin has entailed a rearrangement of the genes. If these two species carry approximately the same number of genes their distribution must be very different.

From a broader viewpoint the evidence which has been presented throws very grave doubt upon the idea that there is necessarily any extensive homology between the chromosomes of eutherian species in general or or even of closely related species. An extensive shifting of material between the chromsomes (such as has taken place in two closely related rodents) taken together with an inversion of a chromosome segment such as STURTEVANT and PLUNKETT have described would very quickly alter the genetic composition of any chromosome. The fact that the eutherian mammals so far studied all show a high number and a general similarity of seriation (in size) does not necessarily mean that there is any great similarity in the genetic make-up of, say, the largest chromosome. The

high number and perhaps the tendency towards a certain type of chromatin distribution may be dependent upon structural peculiarities of the eutherian cells and without any phylogenetic significance as far as the make up of the individual chromosomes is concerned.

SUMMARY

1. From considerations presented and discussed above we should expect the chromosomes of *Mus norvegicus* and *Mus musculus* to be similar. On the contrary they are very different.

2. In *Mus norvegicus* the diploid number is 42 and there are two pairs of large chromosomes nearly double the size of the next largest pair. In *Mus musculus* the diploid number is 40 and these form a closely graded series when placed in serial alinement.

3. The differences noted for the two species are too great to be accounted for on the basis of a breaking in two of a chromosome or the fusion of two pairs. It is concluded, therefore, that since the rat and mouse took their origin from a common stem there has been an extensive shifting of the chromatic material between non-homologous chromosomes, independently, in both species.

4. If the above conclusion is correct, added light is thrown on the problem of the sterility which results from species crosses and, furthermore, grave doubt must be entertained concerning the idea that there is necessarily any extensive homology between the chromosomes of different eutherian species.

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THE DISTURBING EFFECT OF THE GLUTINOUS GENE IN RICE ON A MENDELIAN RATIO[1]

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NATURE OF THE GLUTINOUS CHARACTER AND ITS SIGNIFICANCE

According to texture of endosperm, rice varieties are popularly divided into main groups, namely, starchy and glutinous. The endosperm of the former is hard and corneous and that of the latter is softer and opaque. When boiled, glutinous rice forms a glue-like mass, while in common starchy rice, when this is properly cooked, the grains remain free and entirely distinct. To this property of forming a sticky mass, the glutinous rice owes its name; but the substance responsible for this behavior is a carbohydrate rather than gluten.

From the genetic standpoint, it has been shown, by several investigators whose work will be reviewed presently, that the glutinous character is

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governed by a simple Mendelian recessive gene. This simple recessive factor exerts its influence not only in the endosperm, but also in the pollen in such a way that the carbohydrate reserve material gives a reddish-brown reaction to the iodine reagent instead of the normal blue reaction (PARNELL 1921).

Since it affects the endosperm, the glutinous character is visible sooner than other sporophytic characters. It is readily studied in large numbers, an average panicle bearing several hundred grains. The character is also manifested in the gametophyte generation, so that direct results of Mendelian segregation with regard to the glutinous pair of factors, *Gl gl*, can be observed. Its further significance lies in the fact that it is very similar to the waxy character in maize, which has recently aroused a good deal of genetic interest.

Fom the practical standpoint, glutinous rice is also very valuable, particularly in the Orient. In China, it is universally used in different forms of pastries on several national celebrations, such as the "Lantern Festival," the "Dragon Festival," and others. The straw of glutinous varieties is more pliable and has greater tensile strength than that of starchy rices, qualities highly valued in certain industries. Both the grain and the straw command a premium on the market. Glutinous rice, like the starchy class, is very rich in varieties. Thus, of the 974 classified Philippine varieties, 144 are glutinous. Corresponding figures for Javanese varieties are 756 and 41, according to COPELAND (1924). While the Occident does not raise the glutinous rice to any extent, about 8 percent of the rice area in Japan is planted to this sort and in China the estimate is as high as 20-30 percent.

REVIEW OF LITERATURE

Mendelian characters which are expressed in the endosperm have been found in barley, rye, wheat, and corn. COLLINS (1909) found, in a variety of maize imported from China, a new type of endosperm later known as waxy. Studies on the inheritance of the waxy character by COLLINS and KEMPTON (1911) and KEMPTON (1919) demonstrated that the waxy character behaves as a simple Mendelian recessive and that a significant deficiency of waxy kernels occurs in F_2 when heterozygous individuals are self-pollinated. That the waxy character in maize is also manifested in the pollen grains is shown by BRINK and MACGILLIVRAY (1924), DEMEREC (1924), and LONGLEY (1924). BRINK (1925) and KIESSELBACH and PETERSEN (1926), further found it manifested in the female gametophyte. To account for the deficiency of the waxy kernels, BRINK and MACGILLIVRAY

(1924) suggested a differential rate of pollen tube growth. MANGELSDORF and JONES (1926) postulated that this differential pollen tube growth is due to the presence of an accessory factor linked with the $W_x w_x$ pair rather than to the waxy gene itself.

The glutinous character in rice is similar to the waxy character in maize. The inheritance of this character has been studied by several investigators.

HOSHINO (1902) observed xenia in his crosses between *Oryza glutinosa* and *O. sativa.*

KATO also studied the glutinous character, but I have been able to secure his data only from a review paper by CHANG (1921). KATO's F_2 population consisted of 286 non-glutinous grains and 101 glutinous ones, whereas 290.25 and 96.75 are expected, respectively, on the basis of a 3:1 ratio. This gave a plus deviation of 4.25 glutinous grains which is, however, only 0.74 times the probable error.

In accord with KATO's data, IKENO (1914) obtained a larger excess of glutinous grains. In a total F_2 population of 7916 grains, there is a plus deviation of 2.11 times the probable error. Further analysis of IKENO's data, however, reveals the fact that the results of his reciprocal crosses stand in contrast to each other, as shown in the following table reconstructed from the original data:

	Non-glutinous grains	*Glutinous grains*	*Total*	*Deviation*	$\frac{Dev.}{P.E.}$
Common × glutinous	637	178	815	−25.75	3.08
Glutinous × common	5245	1856	7101	80.75	3.27
Total	5882	2034	7916	55.0	2.11
Expected	*5937*	*1979*			

Here, common × glutinous gave a deficiency of the glutinous type which is over three times the probable error, and the reverse condition happened in the reciprocal cross.

PARNELL's (1921) data show a significant deficiency in the glutinous class. From nine F_1 plants, each giving about the same ratio, he obtained 5292 non-glutinous grains and 1587 glutinous ones, whereas 5159 and 1720 are the expected values. This gives a deviation of −133 individuals, which is 5.49 times the probable error. Here, then, the deficiency is significant for the odds against the occurrence of such a deviation being due to chance alone are several thousand to one.

YAMAGUCHI (1926) studied two cases. In one of these, the ratio of non-glutinous and glutinous grains in F_2 is 3490:925, showing a minus deviation of the recessive class over 9.21 times the probable error. In the other case, the ratio is 1754:709, giving a plus deviation that is 6.47 times the probable error. He attempted to explain the deficiency in the first case on the basis of two factors, *H* and *I*:

1 *HHII* 2 *HHIi* 2 *HhII* 4 *HhIi* 1 *HHii* 2 *Hhii* 1 *hhII*	13 starchy	2 *hhIi* 1 *hhii*	3 glutinous

Factor *H* prevents hydrolysis of the starch reserve, whereas in the absence of *H*, or presence of *h*, the starch is further hydrolyzed and erythrodextrin (?) results. The factor *I* is similar, but is less active than *H* so that two doses of *I* are necessary in order that the carbohydrate remains as starch. The presence of one dose of *I* and two doses of *i* occasions the hydrolysis of the starch into erythrodextrin (?). This dihybrid hypothesis, however, does not explain the excess of recessives in his second case; furthermore, it meets other serious difficulties, one of which is that the glutinous genotype, *hhIi*, should throw starchy grains in the next generation on self-pollination.

The present investigation was started in the fall of 1924. The interest in this problem is threefold. We have sought (1) to arrive at a definite interpretation of the observed facts in terms of the distribution of genetic factors; (2) to clarify the results obtained by the earlier workers on this subject; and (3) we have attempted to discover when and how the germinal elements involved bring about their differential effect.

MATERIAL AND METHODS

In this study, twelve varieties of glutinous and non-glutinous rice were used.

100 A glutinous variety known as "Chhalbe," *Oryza sativa* L., obtained from the MANCHURIAN AGRICULTURAL EXPERMENTAL STATION, in 1925.

200 A non-glutinous variety known as "Han diouza" obtained from the same source as 100.

300 A selection of a glutinous rice, known as C. I. No. 654-1, obtained from Mr. J. M. JENKINS of the RICE EXPERIMENTAL STATION, Crowley, Louisiana in 1924.

400 A pure line known as Fortuna C. I. No. 1344, selected from a non-glutinous variety "Pa Chiam" of Formosa, obtained from the same source as 300.

600 A non-glutinous variety known as "Yu-Tao" or "Chinese Imperial Rice," obtained from Mr. C. C. CHEN of TSING HUA COLLEGE, Peking, China.

700 A non-glutinous variety known as "Ta peh mon" or "big white awned," obtained from Mr. C. Y. PAN of PEKING AGRICULTURAL UNIVERSITY.

800b A non-glutinous strain isolated from a Chinese variety known as "Hei Lo Tao," native to Shen-si Province, China.

900 A glutinous variety grown in Tseng Hua district, Chihli Province, China, obtained from the same source as 700.

1000 A non-glutinous variety known as "Shang-Tao" or scented rice, grown in Shen-si Province, China.

1300 A glutinous variety known as "Acupangna," obtained from Mr. Higgins of the UNIVERSITY OF THE PHILIPPINES.

Using the varieties listed above as parental stocks, many cross-pollinations were made in different combinations, and 573 successful crosses were obtained. F_2 and F_3 generations were raised from these crosses.

An iodine solution was used in distinguishing the glutinous from the non-glutinous character in the pollen and in the endosperm. The glutinous type of pollen and endosperm is stained reddish-brown while the non-glutinous type is stained blue. The formula for the stock solution of iodine is:

I—5 grams
KI—10 grams
H_2O—500 cc.

This stock solution is diluted to the strengths desired.

The method of crossing

Crosses were made reciprocally in different combinations involving all the varieties listed above. In the actual operation of crossing, two methods were used. The first one was a modification of the method suggested by SHARNGAPANI (1924). The palea and lemma were very gently pulled apart with the fingers and the stamens removed with a pair of fine bent forceps. After pollination, the lemma and palea were brought together and tied with a piece of fine silk thread. The first set of crosses between two Manchurian varieties was made by this method and twelve seeds were obtained as a result of over 2000 pollinations. The method was then

abandoned for two reasons: first, the process is terribly slow, and secondly, the tying of each small spikelet with a silk thread is a painstaking act and very often injures the lemma and palea, resulting very frequently in the shriveling of both.

The second method is quicker and more certain of success. Emasculation was done either very early in the morning before sunrise, or late in the afternoon after sunset, the reason being that the pollen sacs are very sensitive to the touch and may suddenly discharge the pollen grains when emasculation is done under bright sunshine. For some varieties, emasculation had to be performed before the panicle emerged from the leaf-sheath, but for others emasculation had to be made later. Since rice flowers mature from the top panicle downward, all the younger spikelets on the lower part of the panicle were cut off, leaving about 10-15 spikelets at the top. When the anthers reached about half the length of the lemma and palea (this can be seen by holding the spikelet against the sunlight), a very short transverse cut was made near the tip of the spikelet with a pair of very fine scissors. The anthers were carefully removed with a very fine pointed, bent forceps through the transverse cut, the forceps being sterilized with 95 percent alcohol after each movement. Then the panicle was bagged with a glacine paper bag. About fifteen to twenty-four hours later, the emasculated flower was ready to be pollinated, the most receptive stage of the stigma being indicated by the rapid elongation of the filaments remaining after the anthers had been removed. Pollination was done by inserting one or more mature anthers of the desired type into the hollow of the spikelet and dusting the pollen grains into it. The pollinated panicle was then rebagged and tagged with all necessary markings. This method has many advantages over the previous one. First, the process is much quicker. Secondly, pollination may be easily repeated several times on the same flower and is, consequently, made much surer. Thirdly, the time of greatest receptivity of the pistil is indicated by the rapid elongation of the filaments. The only disadvantage of this method is that after the spikelet is cut crosswise at the top, the endosperm of the developing grain grows out of the opening and becomes elongated and slender, instead of retaining its natural plump and strongly compressed shape. However, such grains germinate and grow just as well as the normal type. The following table shows the percentage of success for different periods of pollination, indicating that better results were obtained when pollination was done in the morning than in the afternoon.

Time of pollination	*9–10 A.M.*	*10–11 A.M.*	*11–12 A.M.*	*1–2 P.M.*	*2–3 P.M.*	*3–4 P.M.*	*Total*
Number of spikelets pollinated.....	14	336	532	98	325	182	1484
Number of seeds set.....	9	146	228	25	120	45	573
Percent success..	64.28	43.45	42.85	25.51	37.26	24.72	38.61

The method of recording

The method of keeping pedigree records of the crosses and their successive generations may be illustrated by the example, 101205*DSt*98, where 101 = the first plant of the glutinous variety 100; 205 = the fifth plant of the non-glutinous variety 200; 101205 = F_1 plant, 101 being the pistillate parent and 205 the pollen parent; *D* = the fourth plant; *St* = the starchy class; 98 = the 98th F_2 individual. The total expression, 101205-*DSt*98, then, means the 98th F_2 plant of the starchy class which has come from the fourth F_1 plant of the cross between plant No. 1 of the glutinous variety 100 and plant No. 5 of the non-glutinous variety 200, the former being used as pistillate parent and the latter as pollen parent. This method was found to be very practical in application.

The method of classification

The grains were cut open at their distal ends. After cutting, all the grains from the same plant were put in a Petri dish and enough iodine solution of the proper strength was poured into it to submerge the grains. After a minute or two, the solution was drained off and the red-stained glutinous and the blue-stained non-glutinous grains were picked apart with a pair of forceps. The method was found to be very accurate.

The method of vegetative propagation

When the first growth reached the flowering stage, the depth of water was increased and some solution of ammonium sulphate was added to the pot. This increase of water submerged all the lower nodes of old shoots and very soon new shoots arose from such nodes. The application of nutrients hastens the growth of young stoolings. When the latter reached about the flowering stage, the first growth was ready to be harvested. Then the same process was repeated again and a third growth immediately resulted. Thus, several successive crops could be raised from the same plant. In this way, some of the F_1 plants were kept segregating for two

years without altering their genetic constitution, and interesting results were obtained from successive crops in certain particular lines of study.

EXPERIMENTS AND RESULTS

Distribution of glutinous and non-glutinous grains in F_2 from reciprocal crosses between two Manchurian varieties

The first set of crosses was made in the spring of 1925 between the two Manchurian varieties, designated as 100 and 200 respectively, the former being glutinous and the latter non-glutinous. Twelve F_1 plants were secured the following summer, each giving about the same ratio of glutinous and non-glutinous grains in the F_2 population. With the exception of two cases, all the heterozygous plants showed a deficiency in the glutinous class and three of them gave deficiencies over three times the respective porbable errors. Of the two exceptions, one gave a plus deviation of 9.5 glutinous grains and the other only 2.25; neither, therefore, is significant. The data are presented in table 1.

TABLE 1

12 F_2 progenies from 100×200.

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	DEVIATIONS	DEVIATION / P.E.	PERCENT GLUTINOUS	RATIO
Progenies each with a deficiency over 3 times the P.E.						
101205D	397	106	−19.75	3.01	21.07	3.16:0.84
103205A	638	168	−33.50	4.04	20.84	3.17:0.83
104207A	297	68	−23.25	4.16	18.63	3.25:0.75
Total	1332	342	−76.50	6.40		
Progenies with no significant deviation						
101205A	1176	373	−14.25	1.24	24.08	3.04:0.96
101205B	743	234	−10.25	1.12	23.95	3.04:0.96
101205C	1142	378	− 2.0	0.17	24.86	3.01:0.99
101205E	592	171	−19.75	2.44	22.41	3.10:0.90
101205F	426	133	− 6.7	0.907	23.79	3.05:0.95
101205G	599	190	− 7.25	0.88	24.06	3.04:0.96
205101A	382	140	9.5	1.42	26.81	2.93:1.07
103205B	611	172	−23.75	2.9	21.96	3.12:0.88
103205C	693	234	2.25	0.253	25.24	2.99:1.01
Total	6364	2025	−95.70	3.57	24.13	
Grand total	7696	2367	−148.75	5.08	23.52	3.06:0.94

A brief analysis of the data in table 1 reveals three important points. First, each of the twelve segregating plants gives approximately a 3:1 ratio, thus strengthening a previous statement that the glutinous character is due to a simple Mendelian recessive gene. Secondly, there is a total deficiency in the glutinous class about 5.08 times the probable error which appears to be significant, for the odds against the occurrence of such a deviation being due to chance alone are over 1341 to 1. Thirdly, this large deficiency in the totals appears to be due to the accumulation of the ten smaller ones in the same direction. Attention may also be called to the plant 104207A, which gave only 18.63 percent glutinous grains instead of 25.0 percent.

Further data from the successive crops of this plant, however, showed that it produced 26.62 percent and 26.23 percent glutinous grains in the second crop and fourth crop respectively (see table 6). This means that though only 18.63 percent glutinous grains were produced in the first crop, it retained the potentiality for producing the percentage expected on Mendelian grounds. In the light of these facts, it seems to be justifiable to state that the gene in question is somewhat plastic in its function under different conditions and that the glutinous material in the 12 sister progenies recorded in table 1 is in all probability homogeneous.

Comparison of F_2 distribution in successive crops of 100×200

The data presented in table 1 agree very closely with those of PARNELL (1921). This agreement, however, does not imply that the results of other earlier investigators are inaccurate. Indeed, it was through the attempt to find out how the fluctuations of those workers might have arisen that the writer resorted to a new channel of approach. A method was developed, as described previously, of propagating the heterozygous plants by vegetative means. It was thought that with the genetic constitution remaining constant, any difference in the ratio of the glutinous and non-glutinous grains in successive crops raised by vegetative propagation might be attributed to the effect of the environment on the behavior of the gene in question; thus, by comparing the segregation in successive crops, a glimpse might be gained as to how the glutinous gene responds under different conditions. Mention may be made here that during the second growth, five of the twelve F_1 plants, namely, 101205A, 101205C, 101205G, 103205A and 205101A were lost. Eight more crops were raised from the remaining seven plants. The data are presented in table 2.

TABLE 2

Comparison of segregation in successive crops of 100×200.

CROP	TIME OF HARVESTING	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEV. / P.E.	PERCENT GLUTINOUS
First	9/21/25	7696	2367	10063	−148.75	5.08	23.52
Second	10/30/25	852	293	1145	6.75	0.46	25.58
Third	1/18/26	227	87	314	8.5	1.64	27.70
Fourth	4/ 9/26	1466	491	1957	2.75	0.21	25.08
Fifth	5/14/26	439	148	587	1.25	0.17	25.21
Sixth	6/28/26	1373	488	1861	22.75	1.80	26.22
Seventh	7/29/26	1478	464	1942	−21.5	1.74	23.89
Eighth	8/31/26	1141	371	1512	− 7.0	0.61	24.53
Ninth	10/23/26	1457	452	1909	−25.25	1.97	23.67
Total		16129	5161	21290	−161.5	3.79	24.24

Generally, the grain matures about thirty days after pollination. A chronological analysis of table 2 reveals the fact that all pollination in the period beginning from October 1, 1925, to the last of May 1926, resulted in the production of over 25 percent glutinous grains in every case, while all pollinations in the period from the latter part of June to the latter part of September resulted in the reverse. To put it in more explicit terms, the glutinous gene with the reaction of its associates gave rise to the production of 23.52 percent glutinous grains under the conditions prevailing when the first crop was raised; the same genetic complex, however, produced more than 25 percent consecutively in the five succeeding crops raised in the second period and, in the following period, again, less than 25 percent of glutinous grains was produced consecutively in three crops. These orderly periodical changes may be taken to indicate that while the glutinous gene itself was not changed under different conditions, its physiological effect was altered more or less as shown by the end results. While it is entirely conceivable how the earlier investigators got fluctuations in their results in the light of the present data, conclusion in this respect will be deferred until further data are presented.

Distribution of F_2 classes from 14 combinations of unrelated forms

In the summer of 1925, numerous crosses were made involving ten unrelated glutinous and non-glutinous varieties obtained from different countries. As a result, nine F_1 plants were secured from the combination

421×104; fifteen from 600×100; seven from 300×600; four from 1315×619; five from 903×619; five from 300×400; three from 421×1316; and two from each of the following combinations, namely, 903×715, 906×706, 320×705, 306×800b$_4$ and 1000b$_7$ 915. F_2 generations were raised in 1926 from all these combinations. In every case, the non-glutinous was dominant over the glutinous in the hybrid and the two types of grains were segregated out in an approximately 3:1 ratio in the F_2. The results are summarized and presented in table 3.

TABLE 3

Segregation from F_2 populations of 14 combinations of unrelated varieties.

COMBINATIONS	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEV. / P. E.	PERCENT GLUTINOUS	RATIO
100×200	16129	5161	21290	−161.5	3.79	24.24	
421×104	7185	2313	9498	−61.5	2.16	24.35	
4269×4957	4748	1471	6219	−83.75	3.63	23.65	
300×600	1263	373	1636	−36.0	3.05	22.80	
1315×619	445	137	582	− 8.5	1.21	23.54	
903×619	1435	428	1863	−37.75	2.99	22.97	
300×400	1143	338	1481	−32.25	2.87	22.82	
903×713	443	154	597	+ 4.75	0.66	25.80	
906×706	574	163	737	−21.25	2.68	22.12	
320×705	446	132	578	−12.50	1.78	22.83	
421×1316	179	60	239	+ 0.25	0.05	25.06	
1000b$_7$×915	229	75	304	− 1.0	0.19	24.67	
306×800b$_4$	244	72	316	− 7.0	1.34	22.78	
600×100	9580	3005.00	12585	−141.25	4.31	23.87	
Total	44043	13882	57925	−599.25	8.52	23.96	3.04:0.96
Expected	*43443.75*	*14481.25*	*57925*	*0*	*0*	*25.00*	*3:1*

A glance at the data in table 1 and those in table 3 reveals their strong resemblance in regard to the deficiency in the glutinous class, in spite of the fact that the former represents the segregates from twelve plants having the same parents, while the latter involves fourteen combinations of unrelated varieties. Of these fourteen combinations, only two showed a slight plus deviation, namely, 4.75 and 0.25 grains respectively, both being, however, small enough to be negligible. All the rest gave a greater or lesser deficiency. The net deficiency amounts to 599.25 grains. Such a deviation is about 8.52 times the probable error. The odds against the occurrence of a deviation as great as this on the basis of chance alone is over fourteen

million to one. These data, therefore, further strengthen the three cardinal points indicated by the data in table 1.

Corroborative evidence for the accumulation of deficiencies

Attention has already been called to the fact that the large deficieny in the totals is due to the accumulation of many smaller ones in the same direction. This statement is well supported by the data presented in table 1, where each of the nine progenies shows no significant deviation, yet there is a deficiency of 95.7 glutinous grains in the totals which is 3.57 times the probable error. A similar situation is also exhibited in the data presented in table 3. Further corroborative evidence for the accumulation of small deficiencies was obtained in the cross 4269×4957. Five crops were raised by vegetative propagation. None of them gave a large deviation, yet a total deficiency equal to 3.63 times the probable error arises as shown in table 4.

TABLE 4

Accumulation of the deficiency of glutinous grains in the F_2 generation of 4269×4957.

CROP	TIME OF HARVESTING	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEV. / P. E.	PERCENT GLUTINOUS
First	4/26/26	661	190	851	−22.75	2.67	22.32
Second	5/14/26	249	71	320	− 9.0	1.72	22.18
Third	6/24/26	844	243	1087	−28.75	2.88	22.35
Fourth	7/23/26	912	310	1222	4.5	0.44	25.36
Fifth	10/ 8/26	2082	657	2739	−27.75	1.81	23.98
Total		4748	1471	6219	−83.75	3.63	23.65

These data are further supplemented by the results obtained from the sixth crop of the combination 600×100. Of the 14 segregating progenies none had a deviation over 2.5 times the probable error. Yet, the total deficiency of glutinous grains amounted to 4.1 times the probable error as shown in table 5.

Cause of the deficiency of glutinous grains

As in all biological researches, when a phenomenon is observed and its reality is subsequently ascertained in explicit terms, the causal agent is next to be sought for; so it is with the present case. The glutinous and non-glutinous characters behave as a contrasting pair of hereditary traits. Breeding facts presented in the preceding pages have shown that they

TABLE 5

Segregating F_2 progenies showing the accumulation of deficiency.

PLANT NUMBER	STARCHY GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.
Progeny with a deficiency over 3 times the probable error					
61010B	73	13	86	− 8.5	3.13
Progencies with no significant deviation					
610101A	82	21	103	− 4.75	1.60
610101C	151	38	189	− 9.25	2.30
611103A	145	36	181	− 9.25	2.35
611103B	123	34	157	− 5.25	1.43
611103C	24	12	36	3	1.71
611103D	133	34	167	− 7.75	2.05
602103A	104	35	139	0.25	0.07
602103B	124	34	158	− 5.5	1.49
608103A	86	23	109	− 4.25	1.39
608104A	69	24	93	0.75	0.26
608104B	83	25	108	− 2.0	0.65
608104C	90	30	120	0	0
615103A	47	11	58	− 3.5	1.57
615103B	178	54	232	− 4.0	0.89
Total	1439	411	1850	−51.5	4.10
Grand total	1512	424	1936	−60	4.68

are due to a pair of allelomorphic genes, G_l g_l, thus giving a simple Mendelian ratio. The data further indicate, beyond reasonable doubt, that there is a significant deficiency in the recessive or glutinous class. The fact that this large deficiency is due to the accumulation of many smaller ones in the same direction has also been ascertained. The question now, is to determine the casual agent that is responsible for the small deviations which, in turn, lead to the significant deficiency. Attempts have been made through the following channels.

Accuracy of classification

It was thought that the small deviations which lead to a significant deficiency of glutinous grains might be due to misclassification. To search for evidence of this kind, all grains from progeny 101205D, which gave a large deficiency as presented in table 1, were planted for further analysis. Due to the damage by cutting in the first classification, only 434 plants were raised out of the original 503 F_2 grains. When these plants reached the

flowering stage, each was examined in duplicate by staining the pollen with iodine solution. As a result, it was found that all the 87 plants arising from grains which had been put in the glutinous class produced homogenous glutinous pollen, while those plants arising from grains that had been classified as non-glutinous comprised two types. One of these types, of which there were 118 plants, produced homogeneous non-glutinous pollen while the other type comprising 229 plants, segregated for glutinous and non-glutinous pollen, the former stained reddish-brown the latter deep blue. This shows that the classification of the F_2 grains was very accurate. Consequently, the attempt to account for the deficiency of glutinous grains on that basis is fruitless.

Effect of seasonal infertility

Under proper environment, when pollinations are successful, all, or practically all spikelets on the pancile are filled, though a few of them may be empty. When the environment is adverse, however, a great percentage of the spikelets remains completely empty, though all pollen grains are still normal, showing that the empty spikelets, in this case, are due to environmental factors rather than to genetic ones. It was early noted that the temperature and humidity fluctuated very much in the greenhouse where the F_1 plants were grown. Parallel to this fluctuation to temperature and humidity was the fluctuation of the percentage of empty spikelets in successive crops of the same plant raised by vegetative propagation. To those empty spikelets due to unfavorable environmental factors not definitely known the term "seasonal infertility" is here applied. It was thought that the high percentage of seasonal infertility of this kind might effect the percentage of glutinous grains in the opposite direction. Attempts were then made to propagate many F_1 plants by vegetative means and collect the fertile and infertile spikelets in successive crops from the same plant or from the same combination of crosses. These data were tabulated to compare with the percentage of glutinous grains for which the plant or combination of crosses in question was segregating. The results are presented in table for the plant 104207A, table 7 for 421×104, table 8 for 600×100, table 9 for 100×200, for table 10 for 4269×4957, respectively.

Table 6 shows that when the seasonal infertility is 31.85 percent, the glutinous class amounts to 26.62 percent in the second crop. In the fourth crop, when infertility gets as high as 56.88 percent, the percentage of glutinous grains still remains about 26. No emphasis can be laid on the third crop because of the small numbers. While these crops seem to

indicate that the glutinous percentage tends to increase with that of seasonal infertility the sixth crop shows the inconsistency, so no definite correlation is indicated by these data.

TABLE 6

Effect of seasonal infertility on the starchy and glutinous ratio in progeny (104×207)A.

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total fertile				
First	9/21/25	297	68	365			18.63	4.16
Second	10/30/25	124	45	169	79	31.85	26.62	.72
Third	1/18/26	5	4	9	63	67.5	44.44	.63
Fourth	4/ 9/26	194	69	263	347	56.88	26.23	.61
Fifth	5/14/26	50	15	65	23	26.13	23.07	.16
Sixth	6/28/26	194	53	247	165	40.04	21.45	1.79
Seventh	7/29/26	131	43	174	49	21.97	24.71	0.13
Eighth	8/31/26	93	30	123	38	23.60	24.39	0.23
Ninth	10/23/26	1	1	2	8	80.00	50.00	0.38

Table 7 shows that when the percentage of seasonal infertility is below 50, the glutinous percentage is below 25 percent, and that when infertility gets as high as 77.55 percent, the glutinous ratio increases to more

TABLE 7

Effect of seasonal infertility on the starchy and glutinous ratio in progeny (421×104).

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total fertile				
First	11/13/25	420	134	554	399	41.86	24.18	0.65
Second	1/18/26	27	9	36	128	78.04	25.0	0
Third	4/14/26	359	142	501	1731	77.55	28.34	2.56
Fourth	5/14/26	243	80	323	2499	88.55	24.76	0.13
Fifth	6/28/26	1401	429	1830	1954	51.63	23.44	2.28
Sixth	8/ 7/26	1285	397	1682	852	33.62	23.60	1.97
Seventh	9/ 4/26	883	278	1161	465	28.59	23.94	1.27
Eighth	10/23/26	2567	844	3411	3353	49.57	24.74	0.51
Total		7185	2313	9498	11381	54.50	24.35	2.16

than 28 percent. While there is an inconsistent tendency for the parallel increase of the percentage of seasonal infertility and that of glutinous

grains, there is no evidence whatever that high seasonal infertility may cause the deficiency of glutinous grains.

TABLE 8

Effect of seasonal infertility on the starchy and glutinous ratio combination of 600×100.

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total fertile				
First	10/30/25	723	270	993	432	30.31	27.19	2.36
Second	1/18/26	210	78	288	887	75.48	27.08	1.21
Third	4/ 9/26	618	188	806	1010	55.61	23.32	1.62
Fourth	5/14/26	129	41	170	29	14.57	24.12	0.39
Fifth	6/28/26	1400	413	1813	508	21.88	22.77	3.24
Sixth	8/31/26	1512	424	1936	466	19.40	21.90	4.68
Seventh	10/23/26	4988	1591	6579	4238	39.17	24.18	2.26
Total		9580	3005	12585	7570	37.55	23.88	4.31

Table 8 shows that when seasonal infertility is as low as 19 percent, as in the sixth crop, there is a large deficiency of glutinous grains which is very likely a significant deviation. On the other hand, when infertility

TABLE 9

Effect of seasonal infertility on the starchy and glutinous ratio combination of 100×200.

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total Fertile				
First	9/21/25	7696	2367	10063			23.42	5.08
Second	10/30/25	852	293	1145	596	34.23	25.58	0.46
Third	1/18/26	227	87	314	1046	76.91	27.70	1.64
Fourth	4/ 9/26	1466	491	1957	1971	50.17	25.08	0.21
Fifth	5/14/26	439	148	587	131	18.24	25.21	0.17
Sixth	6/28/26	1373	488	1861	732	28.23	26.22	1.80
Seventh	7/29/26	1478	464	1942	477	19.71	23.89	1.74
Eighth	8/31/26	1141	371	1512	441	22.58	24.53	0.61
Ninth	10/23/26	1457	452	1909	1792	48.41	23.67	1.97
Total		16129	5161	21290	7186	25.23	24.24	3.79

reaches 75 percent, as in the second crop, the glutinous class contains as high as 27 percent of the population where 25 percent only is expected in

normal segregation. This again lends no support to the idea that high seasonal infertility causes the deficiency of the recessive type.

Table 9 shows that when the percentage of seasonal infertility is highest (76.91) percent, as is the case in the third crop, the percentage of glutinous grains is also highest (27.7) percent.

TABLE 10

Effect of seasonal infertility on the starchy and glutinous ratio in progeny (4269×4957).

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P. E.
		Non-glutinous	Glutinous	Total fertile				
First	4/26/26	661	190	851			22.32	2.67
Second	5/14/26	249	71	320	214	40.07	22.18	1.72
Third	6/24/26	844	243	1087	2785	71.92	22.35	2.88
Fourth	7/23/26	912	310	1222	3524	74.25	25.36	0.44
Fifth	10/ 8/26	2082	657	2739	2621	48.89	23.98	1.81
Total		4748	1471	6219	9144	59.52	23.65	3.63

Table 10 shows that when the percentage of seasonal infertility is lowest (40.07 percent) the percentage of glutinous grains is also lowest (22.18 percent) as is the case in the second crop. On the other hand, when the percentage of infertility is highest (74.25 percent) the percentage of glutinous grains is also the highest (25.36 percent) in the series. This can not be taken as a mere coincidence because the tendency is manifested in the preceding four tables also. After careful analysis of these data, as presented in the preceding five tables (6-10), no satisfactory evidence is found to support the view that the high percentage of seasonal infertility affects the percentage of glutinous grains in the opposite direction. In the light of these data, it seems to be justifiable to state that it is extremely improbable that high seasonal infertility will give rise to the significant deficiency either by diminishing the union of glutinous gametes, or by eliminating a certain proportion of glutinous zygotes.

On the other hand, the same body of data indicates a strong tendency for the parallel increase of the percentage of seasonal infertility and that of glutinous grains in segregating plants. This parallel increase cannot be taken as a mere coincidence. Although the meaning of this parallel increase is unknown, it is conceivable that whatever the conditions are, that cause the high percentage of seasonal infertility they may be, at the same time, advantageous for the activity of the glutinous gene.

Normality of segregation at microsporogenesis

It was thought that the small deviations that lead to a significant deficiency of glutinous grains might be due to some irregularity of chromosome behavior at sporogenesis causing the production of fewer spores that carry the glutinous gene. Unpublished cytological studies by the author show that the meiotic divisions in the pollen mother cells are normal at least in three of the unrelated varieties, namely, 1000, 200, and 1300, the first being diploid and the last two possibly tetraploid. More convincing evidence for the normality of microsporogenesis, however, came from the study of the glutinous and non-glutinous pollen grains which represent the

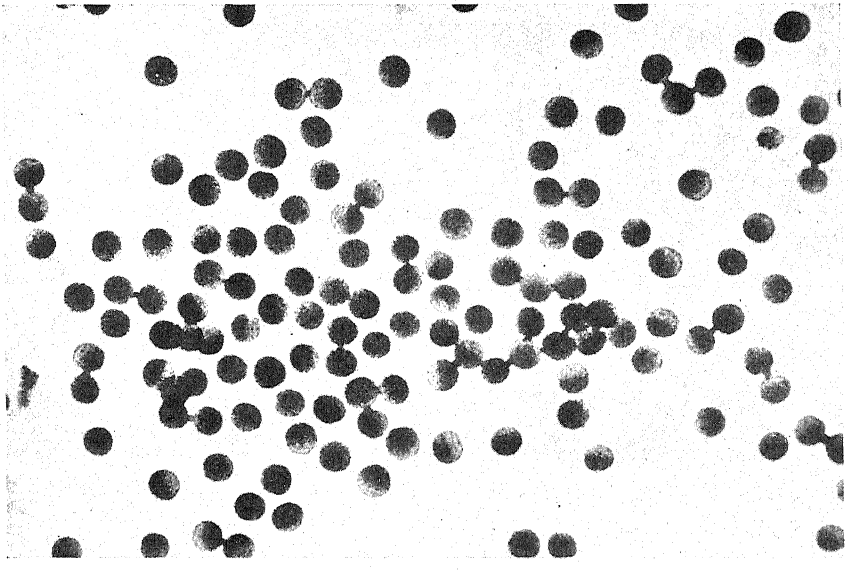


FIGURE 1.—Segregation of non-glutinous and glutinous pollen grains, the former being darker than the latter.

direct results of Mendelian segregation. This was done by counting the two types of pollen, one staining blue and the other reddish-brown in the same anther (figure 1) from segregating plants. The results are presented in table 11.

Of the 6330 pollen grains counted, 3179 were non-glutinous and 3151 were glutinous, while 3165 pollen grains of each type are expected on the basis of a 1:1 ratio. There is a deficiency of fourteen glutinous pollen grains. This deviation is, however, only 0.51 times the probable error. It seems justifiable, therefore, to consider that the two types of pollen are formed with equal frequency.

TABLE 11

Frequency of glutinous and non-glutinous pollen grains.

F_1 PLANTS USED	NUMBER OF ANTHERS EXAMINED	NUMBER OF MICROSCOPIC FIELDS COUNTED	NUMBER OF NON-GLUTINOUS POLLEN GRAINS	NUMBER OF GLUTINOUS POLLEN GRAINS	TOTAL OF EACH TPYE IN THE SAME PLANT	
					Non-glutinous	Glutinous
104207A	1	21	306	318		
	1	12	137	117	443	435
608104A	1	21	180	213		
	1	24	434	405	614	618
608104C	1	25	185	169		
	1	38	230	239	415	408
610101C	1	38	347	325		
	1	36	309	313	656	638
611103A	1	23	222	210		
	1	36	291	287	513	497
608103A	1	25	288	316		
	1	26	250	239	538	555
6 plants	12	325	3179	3151	6330	
Expected			*3165*	*3165*	*6330*	

Possibility of an accessory factor

To account for the disturbed waxy ratio in maize, MANGELSDORF and JONES (1926) advanced the hypothesis that a gametophyte factor, G_a, is present on the waxy chromosome and that this accessory factor disturbs the waxy and non-waxy ratios by causing differential pollen tube growth. Whether this hypothesis is the true explanation for the disturbed ratio in maize, remains to be proven. It is with this hypothesis in mind, however, that the writer further analyzes the situation of the disturbed glutinous and non-glutinous ratio in rice.

It may be pointed out that on the assumption mentioned above, we may have three kinds of combinations in the original crosses in regard to the G_l g_l pair, namely:

1. $G_a\ g_l \cdot G_a\ g_l \times g_a\ G_l \cdot g_a\ G_l$
2. $G_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot g_a\ G_l$
3. $g_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot G_a\ G_l$

If the G_a factor stimulates pollen tube growth, we should expect that from the first combination every F_1 plant ($G_a\ g_l \cdot g_a\ G_l$) will produce more than 25 percent glutinous grains; from the second combination, some F_1 plants will produce an excess of recessives and others a deficiency of recessives; and from the third combination, every F_1 plant ($G_a G_l \cdot g_a g_l$) will produce less than 25 percent of glutinous grains. Since practically all crosses involving many unrelated varieties of rice gave a deficiency of glutinous grains as reported in the preceding pages, the first combination is entirely eliminated and we shall consider the possibility of the last two combinations only. In this consideration, an arbitrary limit is made such that a plus deviation over three times the probable error will be classified as "high recessive" and a minus deviation of the same size as "low recessive," while a deviation less than three times the probable error in either direction will be considered as "normal recessive." It should be pointed out that such a standard is already very low, considering the nature of the problem in hand, although MANGELSDORF and JONES, in their classification, use a smaller deviation (twice the probable error), as the criterion. The justification for such practice will be discussed below.

Evidence from the F_2 *generation.* It was found that among 58 F_1 plants, each producing from two to nine successive crops through vegetative propagation under different environmental conditions, only three plants ever gave an excess of glutinous grains showing a plus deviation over three times the probable error. These were 103205B, 101205B, and 611103D. The data for the plant 103205B are presented in table 12. In its sixth crop,

TABLE 12

Successive crops of 103205B.

CROPS	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
First	611	172	−23.75	2.9	21.96
Second	123	39	− 1.5	0.40	24.07
Third	5	4	1.75	2.01	44.44
Fourth	223	76	1.25	0.24	25.41
Fifth	121	40	− 0.25	0.67	24.84
Sixth	239	106	19.75	3.64	30.72
Seventh	257	74	− 8.75	1.64	22.35
Eighth	235	85	5.0	0.95	26.56
Ninth	512	162	− 6.5	0.85	24.03
Total	2326	758	−13.0	0.80	24.59

30.72 percent glutinous grains were produced. It is to be noted, however, that the same plant produced 21.96 percent of recessives in the first crop showing a deficiency of 2.9 times the probable error. If this plant were really "high recessive," how could such a large deficiency occur in the first crop? Indeed, if only the data from the first crop were availabe, this plant might be classified as "low recessive" according to our standard.

The data for the plant 101205B are presented in table 13. Here, 101205B produced in its sixth crop an excess of glutinous grains giving a plus deviation over six times the probable error. But the number is so small that the deviation can not be considered seriously. This is further indicated by the tendency toward deficiency in the first and fourth crops where the numbers are considerably larger.

TABLE 13

Successive crops of 101205B showing excess of recessives in sixth crop.

CROP	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
First	743	234	−10.25	1.12	23.95
Second	127	39	− 2.5	0.66	23.49
Third	55	18	− 0.25	0.03	24.65
Fourth	208	67	− 1.75	0.36	24.36
Fifth	34	15	2.75	1.34	30.61
Sixth	58	43	17.75	6.03	42.57
Seventh	15	7	1.5	1.09	31.81
Eighth	134	50	4.0	1.01	27.17
Ninth	16	7	1.25	0.89	30.43
Total	1390	480	12.5	0.98	25.66

There remains to be considered the data for the plant 611103D. From the combination of 600×100 fifteen sib F_1 plants were obtained, each of which produced seven successive crops. Only 611103D, however, produced a plus deviation, that is over three times the probable error, as shown in table 14. Furthermore, this excess of recessives was given only in the seventh crop of the plant, as shown in table 15, whereas in its sixth crop, a deficiency of 2.05 times the probable error actually occurred. Indeed, according to the standard of MANGELSDORF and JONES, this plant would have been classified as "low recessive," if we had not obtained further data like that of the seventh crop. On the other hand, the plant, 610101B, producing a slight excess of recessives in the first crop, later gave a significant deficiency of reccessives in the seventh crop, as shown in table 14.

TABLE 14

Sister F_2 progenies from the combination 600×100(7th crop).

PLANT NUMBER	STARCHY GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.
Progeny with an excess over 3 times the probable error					
611103D	306	134	440	24	3.92
Progeny with a deficiency over 3 times the probable error					
610101B	333	77	410	−25.5	4.31
Progenies with no significant deviation					
611103A	184	67	251	4.25	0.91
611103B	56	15	71	− 2.75	1.11
611103C	110	35	145	− 1.25	0.35
610101A	468	139	607	−12.75	1.77
610101C	381	126	507	− 0.75	0.11
602103A	196	72	268	5.00	1.04
602103B	434	134	568	− 8.00	1.14
608103A	275	77	352	−11.00	2.00
608104A	136	35	171	− 7.75	2.03
608104B	236	70	306	− 6.5	1.27
608104C	252	75	327	− 6.75	1.27
615103A	631	200	831	− 7.75	0.92
615103B	990	335	1325	3.75	0.35
Total	4349	1380	5729	−52.25	2.35
Grand total	4988	1591	6579	−53.75	2.26

TABLE 15

Successive crops of 611103D showing excess of recessives in the seventh crop.

CROP	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.
First	47	14	61	− 1.25	0.55
Second	10	3	13	− 0.25	0.24
Third	46	16	62	0.5	0.21
Fourth	..	..	..	..	..
Fifth	64	22	86	0.5	0.11
Sixth	133	34	167	− 7.75	2.05
Seventh	306	134	440	24.00	3.92
Total	606	223	829	15.75	1.87

In the light of these facts, it is justifiable to state that among the 58 F_1 plants studied, there is no positive evidence of one that will produce "high recessives" in the F_2 generation as would be expected from the second combination, $G_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot g_a\ G_l$. On the other hand, the few cases cited like plants 103205B and 611103D, that might have supported the hypothesis with their figures from the sixth crop and seventh crop, respectively, actually offset the excess by the production of deficiencies in other crops.

Evidence from F_3 *generation.* Ten F_1 plants were obtained from the cross 4269×4957 made at the RICE EXPERIMENT STATION, Crowley, Louisiana, in the summer of 1925. From these ten F_1 plants we get a total F_2 population of 6219 grains of which 4748 were non-glutinous and 1471 were glutinous, giving a minus deviation that is 3.63 times the probable error. Ninety-seven large segregating F_3 progenies were obtained and the data are presented in table 16. Of these 97 progenies, two gave an excess of glutinous grains with a deviation over three times the probable error and three produced a deficiency of recessives each with a minus deviation over three times the probable error, while the remaining ninety-two progenies gave no significant deviation. It is to be noted that the progeny CSt_{32}, which gave a minus deviation over three times the probable error had only 126 grains and, therefore, it should not be considered very seriously. Leaving this progeny out for the time being, we have only four progenies to consider, namely, ESt_{47}, FSt_{33}, ASt_{33}, and ISt_{40}. The former two progenies produced "high recessives," while the latter two gave "low recessives."

While these progenies may be cited in favor of the combination, $g_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot G_a\ G_l$, the evidence is very weak, for the ratio of high, normal, and low recessives is 2:92:2. On the basis of chance alone, the probable occurrence of a deviation as great as three times the probable error in 100 trials is 4.30, and we have four cases out of 97. It seems to be justifiable to consider this occurrence to be within the limits of random sampling.

There is no experimental evidence in support of the hypothesis that the deficiency of glutinous grains is caused by an accessory factor linked with the glutinous gene.

In this connection, attention may again be invited to the fact that the small minus deviations of the ninety-two F_3 progenies in table 16 finally lead to one as large as 142 individuals, which is practically three (2.96) times the probable error. It is this sort of constancy in the occurrence o small diviations that seems to be the significant thing in the last analysis.

DISCUSSION

Different from the ordinary blue-staining starch is the "red-staining starch" found in the waxy maize. The latter exists also in other genera of Gramineae. GRIS (1860) found it in *Oryza sativa* var. *glutinosum*. MEYER (1886) found it in *Panicum miliaceum* var. *canditum glutinosum* and *Sorghum vulgare* var. *glutinosum*. KEMPTON (1921) observed it in *Coix lachryma jobi*. It is of theoretical interest that five genera of Gramineae have this type of starch. It must be borne in mind, however, that the indentity of the materials has not been proved in all these cases.

The typical differences between glutinous and common rice starches is found in the iodine reaction, in which the glutinous rice starch shows a red brownish color, while the common rice starch gives a blue color. TANAKA (1907) thinks that the red coloration of the former is due to a characteristic property of the starch itself and denies DAFERT and MEYERS'S contamination theory of amylopectin, erythrodextrin, or special albuminoids. TADOKORO and SATO (1923) find many characteristic differences between the colloidal properties of glutinous and non-glutinous rice starches, but no noticeable differences between their ordinary chemical properties. They conclude, therefore, that the glutinous rice starch and the non-glutinous rice starch are not necessarily different substances, but seem to be only different in degree of polymerization.

Whatever the two types of color reaction may mean, they serve as excellent criteria in the classification of breeding material. When the red-staining glutinous type is crossed with the blue-staining non-glutinous type, the immediate F_1 is fully blue-staining exactly like the non-glutinous parent. When the F_1 plants reach the stage of sporogenesis, two types of pollen (one red-staining and the other blue-staining) are formed with equal frequency. On self-pollinating such individuals, we obtained an F_2 population of 57,925 individuals comprising 44,043 blue-staining and 13,882 red-staining grains, thus giving a ratio of 3.04: 0.96. Since this behavior corresponds so closely with the Mendelian phenomenon of segregation and independent assortment of genes, it is very safe to attribute the non-glutinous-glutinous character to a pair of Mendelian allelomorphs, G_l g_l, On this basis, we should expect 25 percent glutinous grains in F_2, but actually we get only 23.965 leaving a deficiency of 1.035 percent. While in a small population, a deficiency of one percent may not be noticeable, in a population as large as ours, a 1.035 percent deviation means 599.25 individuals, or 8.52 times the probable error. This deficiency is in all probability of biometrical significance, for the odds against the

occurrence of such a deviation on the basis of chance alone is over fourteen million to one. Sufficient data have been brought forward to show that this significant deficiency is due to the accumulation of many small deviations in the same direction. Almost every set of data presented in the preceding pages indicates such a tendency, but table 5 embodies one of the most clear-cut examples. Indeed, it is these small deficiencies that, in the writer's opinion, demand our serious attention in the attempt to account for the significant deficiency in the totals, the reasons being three fold. First, their pre-existence in an almost constant manner foreshadows the end result. Secondly, their tendency to accummulate in the same direction under normal environmental conditions indicates an intrinsic causal agent. Lastly their small size, so small as to appear insignificant in individual cases, indicates that the agent concerned has a very slight differential effect.

In searching for the differential agency which causes the small deviations several possibilities have been considered. Experimental results in the F_3 generation show that the classification of F_2 grains is very accurate. There is little probability, therefore, that the deviations are due to systematic errors in classification.

Numerous counts of pollen grains show that the glutinous and non-glutinous microspores are formed in equal numbers in F_1 plants having the constitution of $G_l\ g_l$. This demonstrates that the process of sporogenesis is normal.

The study of the relation between the percentage of infertile spikelets and that of the glutinous grains in plants segregating for this character sheds no light on the cause of the deficiency. The spikelet is either entirely empty or completely filled. It is to be noted that the infertility in question is due to environmental factors and fluctuates in amount under different conditions. If it were the causal agent of the deficiency of glutinous grains, we should expect that the higher the percentage of seasonal infertility the greater the deficiency. But this is not the case. The percentage of glutinous grains tends to show a parallel increase with the percentage of infertility.

While all these researches have not directly disclosed the causal agent they, by no means, lead us to despair, for they have actually helped to narrow the problem down to a specific stage. The life cycle of a plant in its essential features consists of four phases, namely, sporogenesis, gametophyte development, fertilization, and the ontogeny of the sporophyte. That the two types of microspores in segregating plants are equal in number and filled with carbohydrate reserves shows that the de-

ficiency is not occasioned before or during sporogenesis. The spikelets are either entirely empty, or completely filled with no grains of intermediate character which indicates that the deficiency is not occasioned after fertilization through selective elimination of certain glutinous zygotes during the ontogeny of the sporophyte. The fact that under one set of environmental conditions a given plant may produce a deficiency of glutinous grains and under other circumstances an excess, makes it improbable that selective fertilization is the cause of the deficiency of glutinous grains. By eliminating three of the four phases in the life cycle of a plant, we are led to seek for the cause of the deficiency of glutinous grains in the development of the gametophyte.

As already stated, COLLINS and KEMPTON (1911) and KEMPTON (1919) have obtained a significant deficiency of waxy grains in maize when plants heterozygous for this character are selfed. A similar deficiency occurs when pollen of a heterozygote is applied to the recessive waxy ear, whereas the reciprocal combination results in a small excess of waxy kernels. To account for this difference, BRINK and MACGILLIVRAY (1924) suggest a differential rate of pollen tube growth. BRINK (1925) has furnished some evidence for the slower growth of the waxy pollen tube in his experiments on short and long silks, though this is not corroborated in a later study (BRINK, 1927). In rice where it is not practical to make backcrosses, the significant deficiency of glutinous grains has only been obtained on selfing plants heterozygous for the glutinous character. Utilizing the evidence from the rice, as well as that from maize, we may note the following relations:

A significant deficiency of glutinous grains has been proven beyond reasonable doubt by an enormous body of breeding results. This deficiency has been demonstrated to be due to the accumulation of many small deviations in the same direction. These small deficiencies—so small as to appear negligible in individual cases—have foreshadowed that their causal agent has a very slight differential effect. By tracing through the life cycle of the plant, it is shown that the differential cause in question is in all probability to be sought during the stage of the gametophyte development. In maize, reciprocal backcrosses have shown that it is the male gametophyte that plays the differential role. In the male gametophyte the most vigorous vegetative activity occurs during pollen tube growth. In heterozygous rice plants having the constitution $G_l\ g_l$, there are two types of pollen tubes in competition. In the light of these facts, it is reasonable to postulate that the significant deficiency of glutinous grains is occasioned by the slower growth of the glutinous pollen tube.

Now, to what is the slower growth of the glutinous pollen tube due? In maize MANGELSDORF and JONES (1926) postulated an accessory factor, G_a, on the waxy chromosome pair stimulating the rate of pollen tube growth. Thus, the combination $G_a w_x$ will give excess of recessives, while $g_a\ w_x$ will produce a deficiency of recessives. Ingenious as this hypothesis may appear to be, it is inapplicable to the present case. It has been pointed out that on this assumption, we may have only three kinds of combinations in the original crosses in regard to the $G_l\ g_l$ pair, namely:

1. $G_a\ g_l \cdot G_a\ g_l \times g_a\ G_l \cdot g_a\ G_l$
2. $G_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot g_a\ G_l$
3. $g_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot G_a\ G_l$

The first combination gives only one type of F_1 plants, $G_a\ g_l \cdot g_a\ G_l$, all of which must produce excess of recessives. But this is not the case. The second combination, as a result of crossing over, produces four types of F_1 plants, namely:

F_1 genotypes	*Behavior in F_2 grains*
(A) $G_a g_l \cdot G_a G_l$	Normal recessive
(B) $G_a g_l \cdot g_a G_l$	Excess of recessive
(C) $g_a g_l \cdot g_a G_l$	Normal recessive
(D) $g_a g_l \cdot G_a G_l$	Deficiency of recessive

From the fifty-eight F_1 plants and their F_2 progenies studied, no positive evidence was obtained for the behavior required by the genotype $G_a\ g_l \cdot g_a\ G_l$. Some F_1 plants were obtained, for example, 103205B, which produced 21.96 percent glutinous grains in its first crop and 30.72 percent recessives in its sixth crop. This phenomenon of producing low recessives at one time and high recessives at another by the same plant without germinal change cannot be explained by the hypothesis of MANGELSDORF and JONES. The third combination calls for three types of progenies in F_3, namely, high recessive, normal recessive and low recessive. An examination of ninety-six F_3 segregating progenies showed two progenies giving high recessive, another two giving low recessive, and ninety-two producing normal recessive. While these four progenies may be cited in favor of the hypothesis, they may be attributed to chance equally well, unless further data point to the contrary.

It may be further noted that the F_2 progenies of KEMPTON (1919) gave an average of 23.7 percent waxy, while his F_3 progenies, an average of 24.6 percent waxy. It is the difference between these two generations that leads MANGELSDORF and JONES to postulate their hypothesis of an accessory factor linked with the waxy gene, for they think that the difference can

not be attributed to the "unfavorable influences inherent to the waxy gene itself." This seems, however, to be unnecessary. If the *same rice plant*, for example 103205B, can produce 21.96 percent glutinous grains at one time and 30.72 percent recessive at another without its genotypic constitution being changed, why should we be alarmed when KEMPTON'S F_2 progenies in maize behave differently from his F_3 progenies grown under different environmental conditions over a range of several years! It is true that the difference between the two generations of KEMPTON in maize is 4.3 times the probable error and would be expected as the result of chance only once in about 267 trials. But it is equally true that the difference in the two crops of the same plant (103205B) in rice is, as shown in table 17, 4.74 times the probable error and would be expected as the result of chance only once in about 655 trials. In brief, the results of the F_2 and F_3 generations in rice lend no support to the hypothesis of an accessory factor. In maize, where MANGELSDORF and JONES have reclassified KEMPTON'S results into high recessive, normal recessive, and low recessive groups, the evidence for their conclusion is meagre, and their usage of a deviation two times the probable error as a criterion is of doubtful value.

TABLE 17

The distribution of waxy and non-waxy maize seed in F_2 and F_3 (KEMPTON'S data) as compared with the proportion of glutinous and non-glutinous seed found in the first and sixth crops from a single rice plant (103205B).

	TOTAL SEEDS	RECESSIVE SEEDS	EXPECTED RECESSIVES	DEVIATION	DEV. / P.E.	PERCENT RECESSIVE	PERCENT DIFFERENCE / P.E. DIFFERENCE	ODDS
Maize F_2	77098	18267	19274	−1007	12.4	23.7	4.3	267.2:1
F_3	25329	6235	6332	− 97	2.09	24.6		
Rice 1st crop	783	172	195.75	− 23.75	2.90	21.96	4.74	655.3:1
6th crop	345	106	86.25	19.75	3.64	30.72		

In the writer's opinion, the distorted ratio in rice may very well be attributed to the disturbing effect of the glutinous gene itself. The most vigorous vegetative activity of the male gametophyte is shown during pollen tube growth. If the glutinous gene, g_l, is in a sense weaker in its dynamic activity than its normal allelomorph G_l, it is conceivable that the pollen tube carrying the gene g_l will grow more slowly than the pollen tube carrying G_l. Since our deficiencies are so small in individual cases, any slight differential activity between the allelomorphs, *Gl gl*, will produce the

observed deviations. That these small apparently insignificant deviations lead to a significant deficiency in the long run is also indubitable. The physiological activity of the gene depends in part on its environment. Under one condition, it acts one way and under another condition, it may act differently. This is an observed phenomenon in *Drosophila melanogaster*. The abnormal abdomen character is a case in point. Although the pollen tube carrying the glutinous gene grows more slowly under certain physiological conditions, it may grow faster under another set of environmental factors. This is in full accordance with the experimental data in the case of the F_1 plant, 103205B, which produced 21.96 percent glutinous grains in the first crop and 30.72 percent in the sixth crop. Observations show that excessively high temperature and low humidity contribute to the so-called seasonal infertility. Experimental data indicate that as seasonal infertility increases, the percentage of non-glutinous grains in F_2 decreases in favor of the glutinous type. It is possible that whatever environmental factors, such as temperature and humidity, are unfavorable for the activity of the non-glutinous gene on the one hand, may be favorable for the functioning of the glutinous gene on the other hand. This seems to be clearly indicated by the parallel increase in percentage of glutinous grains "seasonal infertility". In the light of all these facts, it appears justifiable to attribute the distorted glutinous ratio to the disturbing influences inherent in the glutinous gene itself. How the glutinous gene operates in causing different rate of pollen tube growth is an open question. In this connection, it is essential to bear in in mind that rice is an hermaphroditic seed plant which has an alternation of generations. Pollen tube development intervenes between the reduction divisions and fertilization. Here we are dealing with a delicate situation where the problem of heredity and the problem of development are intimately related. BRINK (1925) has well said, "When the 'latitude of genetic indetermination' is relatively wide, our problem becomes as much a physiological as a genetic one."

While the problems of heredity are being worked out satisfactorily, studies concerning the mechanics of development are just beginning. Preliminary study of the composition of glutinous and non-glutinous starch prepared in the pure form from the endosperm of the two types of rice, has shown that the glutinous starch contains about 12.72 percent crude α-amylose and 83.55 percent β-amylose, whereas the non-glutinous starch contains 75.48 percent α-amylose and 17.02 percent β-amylose. It is hoped that through the study of enzyme activity in pedigreed cultures of non-glutinous and glutinous plants in the future, we may learn

more about the physiology of the glutinous gene and its disturbing effect on pollen tube development.

SUMMARY

1. The non-glutinous and glutinous pair of characters is inherited in the Mendelian manner, non-glutinous being apparently completely dominant.

2. Abundant data have been brought forward to prove that they are due to a single pair of genetic factors which are designated as G_l g_l. In an F_2 population of 57,925 individuals, the ratio of non-glutinous grains is 3.04:0.96. YAMAGUCHI's dihybrid hypothesis is considered invalid.

3. It has been demonstrated beyond reasonable doubt that there is a significant deficiency of the recessive type as the result of self-pollinating heterozygous plants.

4. On the basis of a 3:1 ratio, the deviation from the expected percentage of glutinous grains in the total F_2 population of fourteen combinations is 8.52 times the probable error, and would be expected as the result of chance alone only once in about fourteen million trails.

5. Sufficient data have shown also that the significant deficiency in the totals is due to the accumulation of many smaller deviations in the same direction, each of which may be so small in the individual case as to escape notice.

6. F_3 tests have shown that the deficiency is not caused by wrong classification.

7. Pollen counts have demonstrated that segregating plants produce non-glutinous and glutinous pollen grains in equal numbers, showing that the deficiency of the recessive class is not due to any irregularities at sporogenesis.

8. That the same heterozygous plant which produces a deficiency at one time may produce an excess at another time makes it improbable that selective fertilization or gametic incompatibility is the cause of the deficiency of glutinous grains.

9. Nor can the deficiency be attributed to seasonal infertility, because experimental evidence is brought forward showing that as seasonal infertility increases the proportion of glutinous grains produced also increases.

10. The fact that the spikelets are either completely filled or entirely empty without intermediate types of defective grains is taken to indicate that the deficiency is not occasioned by zygotic lethals.

11. By tracing through the life cycle of the plant, it is shown that the cause of the deficiency is probably to be sought during the development of the male gametophyte. It is postulated that the exact stage is during the vegetative growth of the pollen tube.

12. An accessory factor hypothesis similar to that MANGELSDORF and JONES have advanced to account for the waxy deficiency in maize is not applicable to the present case in rice.

13. The primary cause of the differential pollen tube development which occasions the distorted non-glutinous and glutinous ratio is attributed to the influences inherent to the glutinous gene itself.

14. Experimental evidence has been brought forward indicating that pollen tubes carrying the glutinous gene and growing slower under ordinary conditions, may grow faster under particularly favorable conditions such as temperature and humidity, thus producing an excess of recessives.

15. On the assumption that environmental factors can influence the effect of the glutinous gene on the rate of pollen tube development, the discrepancies observed by earlier investigators may be accounted for.

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APPENDIX

TABLE 16

Ninety-seven F_3 progenies from the cross 4269×4957 segregating for the glutinous character.

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
Progenies with excess over 3 times the probable error						
EST 47	245	105	350	17.5	3.20	30.0
FST 38	349	143	492	20.0	3.08	29.07
Sub-total	594	248	842	37.5	4.42	..
Progenies with deficiency over 3 times the probable error						
AST 33	343	89	432	−19	3.13	20.60
CST 32	114	12	126	−19.5	5.94	9.52
IST 40	226	52	278	−17.5	3.59	18.71
Sub-total	683	153	836	56.0	6.63	..
Progenies with no significant deviation						
AST 13	197	74	271	6.25	1.29	27.31
AST 20	298	107	405	5.75	0.97	26.42
AST 28	656	215	871	− 2.75	0.31	24.68
AST 34	255	97	352	9.0	1.64	27.56
AST 35	170	48	218	− 6.5	1.50	22.02
BST 18	201	72	273	3.75	0.77	26.38
BST 20	284	82	366	− 9.5	1.69	22.40
BST 21	276	86	362	− 4.5	0.80	23.76
BST 22	315	109	424	3.0	0.49	25.71
BST 26	193	62	255	− 1.75	0.37	24.31
BST 30	270	75	345	−11.25	2.07	21.74
BST 39	158	56	214	2.5	0.58	26.17
BST 43	243	79	322	− 1.5	0.28	24.53
CST 1	110	38	148	1.0	0.28	25.68
CST 6	155	51	206	− 0.5	0.11	24.75
CST 11	87	27	114	− 1.5	0.48	23.68
CST 17	95	35	130	2.5	0.75	26.92
CST 22	167	53	220	− 2	0.46	24.09
CST 23	120	35	155	− 3.75	1.03	22.58
CST 24	122	39	161	− 1.25	0.33	24.22
CST 25	156	47	203	− 3.75	0.9	23.15
CST 43	168	56	224	0	..	25.00
CST 51	225	74	299	− 0.75	0.14	24.75
CST 52	125	37	162	− 3.5	0.94	22.84
CST 53	237	72	309	− 5.25	1.04	23.30
CST 56	147	49	196	0	..	25.00
CST 60	223	70	293	− 3.25	00.65	23.89
DST 16	262	88	350	0.5	0.09	25.14
DST 18	190	50	240	−10.0	2.21	20.83

TABLE 16 (continued)

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
Progenies with no significant deviation						
DST 32	231	87	318	7.5	1.43	27.36
DST 33	315	99	414	− 4.5	0.76	23.91
DST 38	209	67	276	− 2.0	0.41	24.27
DST 39	483	136	619	− 8.75	1.20	21.97
DST 40	272	93	365	1.75	0.31	25.48
DST 41	233	81	314	2.5	0.48	25.80
DST 56	275	77	352	−11.0	2.00	21.88
DST 57	255	88	343	2.25	0.41	25.65
DST 59	280	89	369	− 3.25	0.57	24.12
DST 62	177	54	231	− 3.75	0.84	23.38
EST 2	238	91	329	8.75	1.65	27.66
EST 16	224	67	291	− 5.75	1.16	23.02
EST 17	150	63	213	9.75	2.28	29.58
EST 19	250	83	333	− 0.25	0.04	24.92
EST 26	168	45	213	− 8.25	1.93	21.12
EST 28	193	69	262	3.5	0.74	26.34
EST 36	240	101	341	15.75	2.92	29.62
EST 43	268	71	339	−13.75	2.55	20.94
EST 45	161	55	216	1.0	0.23	25.46
EST 46	221	58	279	−11.75	2.40	20.79
FST 8	216	70	286	− 1.5	0.3	24.47
FST 14	196	64	260	− 1.0	0.21	24.61
FST 17	172	49	221	− 6.25	1.2	22.17
FST 21	197	68	265	1.75	0.36	25.66
FST 23	333	91	424	−15.0	2.49	21.46
FST 42	158	55	213	1.75	0.41	25.82
FST 44	129	40	169	− 2.25	0.59	23.67
FST 48	172	47	219	− 7.75	1.79	21.46
FST 49	432	125	557	−14.25	2.06	22.44
FST 51	209	84	293	10.75	2.15	28.67
FST 58	105	35	140	0	. .	25.00
FST 64	249	86	335	2.25	0.42	25.67
FST 65	152	55	207	3.25	0.77	26.57
GST 7	198	67	265	0.75	0.15	25.28
GST 9	218	76	294	2.5	0.49	25.85
GST 11	192	50	242	−10.5	2.31	20.66
GST 21	257	90	347	3.25	0.59	25.94
GST 36	220	58	278	−11.5	2.36	20.86
GST 45	241	76	317	− 3.25	0.62	23.97
GST 48	153	68	221	12.75	2.93	30.77
GST 49	194	72	266	5.6	1.17	27.07
IST 9	236	82	318	2.5	0.48	25.75
IST 10	240	76	316	− 3.0	0.57	24.05

TABLE 16 (continued)

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
Progenies with no significant deviation						
IST 13	271	92	363	1.25	0.22	25.34
IST 15	199	51	250	−11.5	2.49	20.40
IST 17	248	81	329	− 1.25	0.23	24.62
IST 23	300	86	386	−10.5	1.84	22.28
IST 33	279	83	362	− 7.5	1.34	22.93
IST 49	246	76	322	− 4.5	0.85	23.6
IST 60	237	76	313	2.25	0.43	24.28
IST 74	359	121	480	1.0	0.15	25.21
JST 4	201	67	268	0	0	25.00
JST 25	313	124	437	14.75	2.41	28.37
JST 35	248	80	328	− 2.0	0.37	24.40
JST 43	204	75	279	5.25	1.07	26.88
JST 59	232	59	291	−13.75	2.76	20.27
JST 62	199	73	272	5.0	1.03	26.84
JST 64	256	86	342	− 0.5	0.09	25.14
JST 69	157	40	197	− 9.25	2.25	20.30
JST 73	331	103	434	− 3.0	0.49	23.73
JST 82	232	73	305	− 3.25	0.63	23.93
Sub-total	20329	6587	26916	−142.0	2.96	24.47
Grand total	21606	6988	28594	−160.5	3.23	24.44

BIONOMIC STUDIES ON CERTAIN TELEOSTS (Poeciliinae). II. COLOR PATTERN INHERITANCE AND SEX IN *Platypoecilus maculatus* (Günth.)

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The fishes upon which the accompanying observations were made were described in Study I of this series (BELLAMY 1924). Also a preliminary account of the hereditary behavior displayed by them was given at the Toronto meetings of the American Society of Zoologists, 1922 (See Anatomical Record, 1924, pp 419–420). Since that time enough data have accumulated to make it reasonably certain that the interpretation previously indicated is the correct one, namely, that the color varieties named Nigra (*N*), Pulchra (*P*), Rubra (*R*), and White (*W*) constitute a multiple allelomorphic series of sex-linked characters, with the female heterogametic for sex factors. Males may be heterozygous for any two but not more than two of the factors for these characters. Females carry only one. Hence males may be described as *NN*, *NP*, *NR*, *NW*, *PP*, *PR*, *PW*, *RR*, *RW*, and *WW*; females as *NO*, *PO*, *RO*, and *WO*.

In the accompanying pedigree the animals are represented by these symbols and while they serve to indicate the interpretation just mentioned, since they also describe the color and sex of the fishes so recorded, the data can be compared with any hypothesis the reader may have in mind.

GORDON (1927) whose paper came to my attention some time after this report was in manuscript, objects to certain of the above statements. He suggests "stippled" as a more suitable name for "White." His term more accurately describes the (microscopic) pattern of the fish but not the general appearance of the animal as one sees it with the unaided eye.

While I did not consider a factor for stippled under that name the existence of the small melanophores characteristic of the white platypoecilus was not ignored (BELLAMY 1924, page 523). So far as the description of hereditary behavior in the four varieties here described is concerned either term may be used.

GORDON seems to think that the factors *W*, *N*, *P*, *R*, do not constitute a multiple allelomorphic series, meaning, I suppose, that *W* does not belong in the series. Perhaps it does not. The data presented must speak for themselves.

Objection is raised to the statement that "males may be heterozygous for any two of the four characters, *W*, *P*, *R*, or *N*; and the female may carry the determiners for only one". So far as my experience goes the statement is true as it stands, and if one may resort to what approaches hear-say evidence, so far as I am aware none of the numerous Chicago fanciers ever obtained a Rubra-Pulchra, Rubra-Nigra, or Nigra-Pulchra

TABLE 1

White male *WW* × Colored female *CO*	
Colored males *CW*	White females *WO*
F_1 127	116

F_2 Colored males *CW*	White males *WW*	Colored females *CO*	White females *WO*
46	74	70	83

female, although to my knowledge they attempted it repeatedly. Further it is not clear from GORDON's data (his table 2 and pages 268–270) that he had a pulchra-rubra female.

TABLE 2

Colored male *CC* × White female *WO*	
Colored males *CW*	Colored females *CO*
347	358

Colored males *CC* *CW*	Colored females *CO*	White females *WO*
411	289	258

Crosses involving Golden and Rubra as well as Nigra, Pulchra and White were made in 1923–24 and further discussion of GORDON's data can be undertaken more profitably in connection with my own data on the same types of crosses.

It will be sufficient here to add that White (stippled) whether sex-linked or not is used in exactly the same sense as "wild-type" in Drosophila.

There is not the slightest difficulty in separating the four color varieties from one another but certain of the heterozygous males are not readily distinguished. *PP* and *PR* look much alike until they are fully

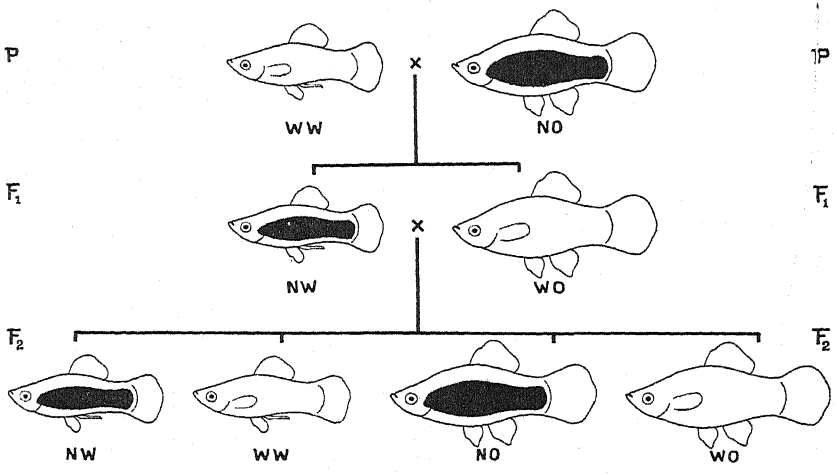


FIGURE 1.

mature when the red color and heavier pigmentation of *PR* enables one to distinguish most of them. In progenies where both *NP* and *PP* or *PR* appear there are usually a few males that are not readily distinguish-

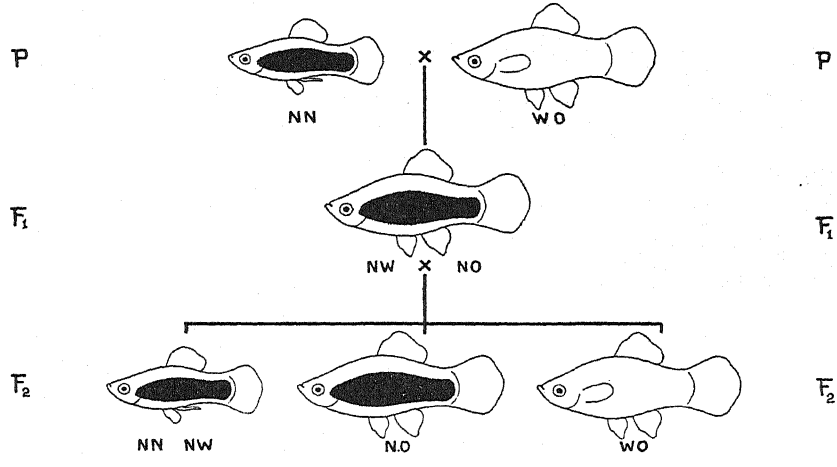


FIGURE 2.

able. Also since white is recessive to all of the other color characters, *NW*, *PW*, and *RW* must be tested by breeding. Obviously, however, in pedigree material the type of mating enables one to know whether the males

will be heterozygous for *W*. For the most part we avoid those matings that give combinations difficult to separate accurately.

As far as possible all of the relevant information concerning the hereditary behavior of these four color varieties is included in the accompanying pedigree. In the first column which describes the mating, males are indicated on the left of the $\times$. In several cases the same male is used in more than one mating. This is indicated by a sub-numeral following the descriptive formula of the male, the same sub-numeral being used in all of the matings in which that particular male appears. The NN_3 male in mating 261 is the same individual that appears in mating 31.2.3; the male NR_9 is used in matings 273, 272, and 217.2. The second column in the pedigree gives the original record number, and in the third and fourth columns the source of the male and female parent is indicated. The number of young born in each mating is given in the fifth column and in the following 18 columns the color and sex of the offspring recorded for each mating. In the two last columns the number and percentage of offspring not recorded is given.

No evidence of crossing over has yet come to light so that unless the four characters are multiple allelomorphs, as we assume, they must be very closely linked. At any rate the type of hereditary behavior can be seen to good advantage by grouping the matings according to the scheme used in tables 1 and 2—which represent the F_1 and F_2 generations of reciprocal crosses involving a sex-linked characters. (See also figures 1, 2). *NN*, *NW*, *NO*, *PP*, *PW*, *PO*, *RR*, *RW*, *RO*, *NP*, *PR*, *NR*, are classified as "colored". A white male when crossed with a colored female produces colored sons and white daughters. Combining the results of mating 25, 262, 263, 268, and 291 we have in F_1 127 colored sons and 116 white daughters; in F_2 (matings 83, 263.1, 268.1, 314, and 327) 46 colored and 74 white males, 70 colored and 83 white females (table 1). The reciprocal crosses (table 2) give in F_1 all colored offspring—347 males and 358 females (matings 31, 217.2, 255, 259, 265, 286, 289, 290, 298, and 316). In F_2 (matings 21, 21.1, 21.2, 31.1, 31.2, 44, 44.2, 44.2.1, 82, 83.1, 83.2, 89, 89.1, 89.1.1, 217, 217.2.1, 217.2.2, 217.2.3, 259.1, 269.2, 265.1, and 289.1) there are 411 colored males, 289 colored females and 258 white females. In addition there appeared 4 white males, 3 in mating 44.2.1 and 1 in mating 89.

The three white males (44.2.1) appeared in the first brood of 39 young and were isolated as males when 128, 149, and 157 days old. The white male in 89 was about 8 months old when isolated.

It is likely that these 4 males were in some way introduced into the tanks by accident. Although we exercise great care to prevent contamination it sometimes occurs. For example matings 314 and 327 were made to test males apparently out of place where they were first discovered. The male *PW* tested in mating 314 was found in the third brood of young from mating 258. It happened that the second brood of mating 263 was born on the same day. Since these were the only other young born near the date of birth of the *PW* male and since some one in the laboratory, about this time adopted a large bulb pipette for counting the new born young and transferring them to a new tank I feel justified, with this explanation, in including this *PW* male in the records of mating 263 instead of 258. The male tested in mating 327 was found in the third brood of young from mating 272. It is likely that he belongs with mating 268, young from which were born the same day. Needless to say the use of hot water for rinsing the pipette was strictly enforced after these two experiences.

While it is probable that the 4 white males found in the progenies of matings 44.2.1 and 89 are the result of contamination it is possible that they represent crossovers of some sort. Possibly they are a consequence of non-disjunction in the mothers, although one would infer from the heterogametic nature of females—if one may infer at all that the fertilization of a non-disjunctional gamete would result in a female. Perhaps these males represent an inversion of sex in some of the white females, an occurrence for which there is some circumstantial, but no direct, evidence.[1]

In the two matings, 44.2 and 83.1, there is some departure from expected ratios. In the former the male is regarded as heterozygous for the reason that one of his sons, the one mated in 44.2.1 was certainly heterozygous. However the number of recorded young is so small that little significance attaches to the absence of one expected phenotype. But in mating 83.1 where the male must be heterozygous because of his ancestry, it is curious that among the 26 recorded young there should have been no white females. The case is made somewhat more puzzling by the fact that all of the sons of the 83.1 male that were tested proved to be homozygous. It will be seen that a brother of this male (mating 83.2) was heterozygous.

[1] ESSENBERG (1926) has described "complete sex-reversal" in a closely related species, *Xiphophorus helleri*. Two individuals are said to have given birth to young as females and later to have been the fathers of young. It is highly desirable that these very interesting observations be repeated, under circumstances where they can be kept under continuous observation. (See page 99 of ESSENBERG's paper).

With reference to the possibility of the male in 83.1 being a result of contamination it should be said that until the offspring from matings 82 and 83 were born the only pulchras in the entire laboratory were the two males used as fathers in these two matings. By assuming crossing over in the male *PW* used in 83 or even in his son, used in 83.1 and invoking the aid of homozygous lethals it is not particularly difficult to make a restatement in substantial agreement with the absence of white females in 83.1. If, for instance, we assume that instead of being allelomorphs *P* and *W* are very closely linked and that the male used in 83.1 resulted from a cross over gamete in the 83 male, then the 83.1 male would have genes for both *P* and *W* in one X chromosome, the other X chromosome being without genes for these characters and therefore, let us say, giving rise to a nonviable gamete, or perhaps, when fertilizing a *W*-carrying gamete producing a non-viable zygote. If all this is true then a number of the males in the "83" line of descent are of the composition (*PW*)*X*-(*P*)X and the females (*PW*)X-Y. I have no real evidence bearing on the matter and until another case appears speculation does not appear to be particularly fruitful.

Attention should be called to mating 89. This female was originally mated to an F_2 hybrid male from a cross of *Xiphophorus helleri* male *Platypoecilus maculatus* (*nigra*) female. The record bears the notation "female may not be virgin" a statement that is borne out by the kind of of young she produced. It is highly probable that the male parent of the young in this mating is a *NW* brother of the female the only type of male, other than the hybrid mentioned, to which she could have been exposed. The mating appears in the pedigree as though the *NW* male had been used in the original mating.

The female used in mating 100 was without an anal fin. She was discovered when about 4 weeks old. All of the F_1 and F_2 young were normal.

In mating 261 the first male, *NN* from 31.2, died 27 days after the mating was made. Fifty-four days later a new male *NN* from 31.2.6.2 was added. The first young were born 71 days after the introduction of the second male. Undoubtedly he is the father of the young in mating 261.

While the inheritance of color pattern in Platypoecilus is clearly of the poultry-type there are no data available for profitable discussion of any possible or probable association between the characters described and sex chromosomes. It would be the obvious thing to predict a ZZ, WZ chromosome equipment.

AIDA (1921) describes a type of sex-modified hereditary behavior in *Aplocheilus latipes*, and oviparous species, very similar to that described

for *Lebestes reticulatus* by WINGE (1922). In both cases males are described as being heterogametic for sex factors and certain genes are associated with the Y-chromosome to account for a father-to-son type of inheritance. In *Lebestes* the problem is complicated by the fact that color pattern characters are strictly sex-limited, appearing only in males. In spite of the fact that it was not possible to establish any morphological differences between X and Y chromosomes in Lebestes and that, further, WINGE has not "succeeded in laying down any morphological or other differences in the chromosomes, which render it possible to distinguish the sex-chromosomes from the autosomes", he takes the position that his data "prove" the existence of X- and Y-chromosomes.

In the light of present knowledge it does not seem easy to construct a mechanism that will satisfy both the Lebestes—Aplocheilus and Platypoecilus types of sex-modified hereditary behavior. Nor for that matter is there any *a priori* reason for assuming that they need be identical. It is quite possible that, as in certain Lepidoptera among insects, these species of fishes, although fairly closely related, have evolved mechanisms of sex determination and inheritance that exhibit some detailed differences.

SUMMARY

A multiple allelomorphic series of four sex-linked color-pattern characters in *Platypoecilus maculatus* Günth. is described. The female is heterogametic for sex factors.

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TABLE 3
Pedigree

MATING	RECORD NUMBER	SOURCE OF PARENTS		YOUNG BORN	NN	NN NW	NW	NO	PP	PP PW	PW	PO	RR	RR RW	RW	RO	WW	WO	NP	NP PR	PR	NR	NOT RECORDED	PERCENT NOT RECORDED
		Male	Female																					
$RW_1 \times RO$	21	Unknown	Unknown	20	..	..	..	..	..	..	..	..	..	5	..	2	..	5	..	..	..	..	8	40
$RW \times RO$	21.1	21	21	3	..	..	..	..	..	..	..	..	..	1	..	1	..	1	..	..	..	..	0	0
$RW_1 \times RO$	21.2	Unknown	21	139	..	..	..	..	..	..	..	..	..	28	..	45	..	35	..	..	..	..	31	22
$WW_2 \times PO$	25	Unknown	Unknown	2	..	..	..	..	..	..	2	..	..	..	..	..	..	0	..	..	..	..	0	0
$NN \times NO$	30	Unknown	Unknown	45	21	..	..	23	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	2
$NN \times WO$	31	Unknown	Unknown	48	..	..	9	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	33	69
$NW \times NO$	31.1	31	31	64	..	14	..	11	..	..	..	..	..	..	..	..	..	25	..	..	..	..	14	22
$NW \times NO$	31.2	31	31	87	..	26	..	18	..	..	..	..	..	..	..	..	..	21	..	..	..	..	22	25
$NN_3 \times NO$	31.2.3	31.2	31.2	61	25	..	..	29	..	..	..	..	..	..	..	..	..	..	..	..	..	..	7	11
$NN \times NO$	31.2.6	31.2	31.2	8	4	..	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0
$NN_4 \times NO$	31.2.6.2	31.2.6	31.2.6	85	39	..	..	36	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10	12
$NN \times NO$	31.4.1	31.4	31.4	67	7	..	..	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	54	81
$WW_2 \times WO$	35	Unknown	Unknown	6	..	..	..	..	..	..	..	..	..	..	..	..	2	3	..	..	..	..	1	17
$RW \times RO$	44	Unknown	Unknown	80	..	..	..	..	..	..	..	..	..	25	..	23	..	7	..	..	..	..	25	31
$RR \times RO$	44.1	44	44	232	..	..	..	..	..	..	..	..	35	..	..	69	..	..	..	..	..	..	128	55
$RW \times RO$	44.2	44	44	20	..	..	..	..	..	..	..	..	..	3	..	7	..	0	..	..	..	..	10	50
$RW \times RO$	44.2.1	44.2	44.2	87	..	..	..	..	..	..	..	..	..	12	..	16	3	11	..	..	..	..	45	52
$RR \times RO$	44.3	44.1	44.1	75	..	..	..	..	..	..	..	..	15	..	..	23	..	..	..	..	..	..	37	51
$RR_5 \times RO$	44.3.2	44.3	44.3	54	..	..	..	..	..	..	..	..	18	..	..	29	..	..	..	..	..	..	7	13
$RR \times RO$	44.4	44.1	44.1	10	..	..	..	..	..	..	..	..	5	..	..	5	..	..	..	..	..	..	0	0
$WW_2 \times WO$	46	Unknown	Unknown	40	..	..	..	..	..	..	..	..	..	..	..	..	16	14	..	..	..	..	10	25
$RR \times RO$	74	Unknown	Unknown	30	..	..	..	..	..	..	..	..	12	..	..	12	..	..	..	..	..	..	6	20
$PW \times NO$	82	25	30	31	..	..	9	..	..	..	..	7	..	..	..	..	..	6	5	..	..	..	4	13
$PW \times WO$	83	25	35	38	..	..	..	..	..	..	7	9	..	..	..	..	5	17	..	..	..	..	0	0
$PW \times PO$	83.1	83	83	34	..	..	..	..	..	15	..	11	..	..	..	..	..	0	..	..	..	..	8	23
$PW \times PO$	83.2	83	83	184	..	..	..	..	..	45	..	21	..	..	..	..	..	19	..	..	..	..	99	54
$PP_6 \times PO$	83.1.2	83.1	83.1	83	..	..	..	..	36	..	..	35	..	..	..	..	..	..	..	..	..	..	12	14
$PP \times PO$	83.1.3	83.1	83.1	59	..	..	..	..	27	..	..	22	..	..	..	..	..	..	..	..	..	..	10	17
$PP \times PO$	83.1.3.1	83.1.3	83.1.3	90	..	..	..	..	13	..	..	12	..	..	..	..	..	..	..	..	..	..	65	72
$NW \times NO$	89	31	31	92	..	24	..	19	..	..	..	..	..	..	..	..	1	13	..	..	..	..	35	38
$NW \times NO$	89.1	89	89	37	..	13	..	6	..	..	..	..	..	..	..	..	..	6	..	..	..	..	12	32
$NW \times NO$	89.1.1	89.1	89.1	85	..	32	..	19	..	..	..	..	..	..	..	..	..	22	..	..	..	..	12	14
$WW \times WO$	100	83	83	24	..	..	..	..	..	..	..	..	..	..	..	..	2	7	..	..	..	..	15	63
$WW \times WO$	117	46	21.1	47	..	..	..	..	..	..	..	..	..	..	..	..	17	18	..	..	..	..	12	26
$WW \times WO$	117.1	117	117	92	..	..	..	..	..	..	..	..	..	..	..	..	8	4	..	..	..	..	80	87
$WW \times WO$	117.1.1	117.1	117.1	104	..	..	..	..	..	..	..	..	..	..	..	..	13	4	..	..	..	..	87	84
$WW \times WO$	188	46	31.1	131	..	..	..	..	..	..	..	..	..	..	..	..	30	54	..	..	..	..	47	36
$WW \times WO$	188.1	188	188	97	..	..	..	..	..	..	..	..	..	..	..	..	32	39	..	..	..	..	26	27
$WW_7 \times WO$	188.1.1	188.1	188.1	54	..	..	..	..	..	..	..	..	..	..	..	..	15	24	..	..	..	..	15	28
$WW_8 \times WO$	188.1.2	188.1	188.1	12	..	..	..	..	..	..	..	..	..	..	..	..	9	3	..	..	..	..	0	0
$RW \times NO$	217	21.2	89.1	18	..	..	5	..	..	..	..	..	..	..	..	7	..	4	..	..	..	2	0	0
Group	217.1	217	217	36	..	..	2	7	..	..	..	..	..	12	..	6	..	5	..	..	..	4	0	0
$NR_9 \times WO$	217.2	217	217	126	..	..	22	21	..	..	..	..	..	..	24	32	..	..	..	..	..	..	27	21
$NW \times RO$	217.2.1	217.2	217.2	164	..	..	..	29	..	..	..	..	..	..	30	..	..	27	..	..	..	11	67	41
$RW \times NO$	217.2.2	217.2	217.2	93	..	..	14	..	..	..	..	..	..	..	..	20	..	17	..	..	..	24	18	19
$NW \times RO$	217.2.3	217.2	217.2	88	..	..	..	17	..	..	..	..	..	..	22	..	..	25	..	..	..	16	8	9
$NN \times RO$	254	31.1	21	27	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	2	23	85
$NR_{10} \times NO$	254.1	254	254	120	11	..	..	27	..	..	..	..	..	..	..	21	..	..	..	..	..	20	41	34
$NN \times RO$	254.1.1	254.1	254.1	33	..	..	..	8	..	..	..	..	..	..	..	..	..	..	..	..	..	12	13	39
$NR_{10} \times WO$	255	254	188	113	..	..	14	27	..	..	..	..	..	..	20	21	..	..	..	..	..	..	31	27
$RR_{11} \times NO$	257	44.3	31.41	123	..	..	..	..	..	..	..	..	..	..	..	45	..	..	..	..	..	67	11	9
$RR_{12} \times NO$	258	44.3	89.1	144	..	..	..	..	..	..	..	..	..	..	..	56	..	..	..	..	..	54	34	24
$NR \times RO$	258.1	258	258	143	..	..	..	16	..	..	..	..	9	..	..	29	..	..	..	..	..	20	69	48
$RR \times WO$	259	44.3	188	195	..	..	..	..	..	..	..	..	..	..	67	..	..	71	..	..	..	57	29
$RW \times RO$	259.1	259	259	32	..	..	..	..	..	..	..	..	..	4	..	2	..	3	..	..	..	..	23	72
$RW \times RO$	259.2	259	259	22	..	..	..	..	..	..	..	..	..	1	..	2	..	4	..	..	..	..	15	68
$NN_8 \times PO$	261	31.2	83.1.2	40	..	..	..	20	..	..	..	..	..	..	..	..	..	..	11	..	..	..	9	22
$WW_7 \times NO$	262	188.1	31.2.3	95	..	..	43	..	..	..	..	..	..	..	..	..	..	47	..	..	..	..	5	5
$WW_8 \times PO$	263	188.1	83.1.2	74	..	..	..	..	..	..	..	36	..	..	..	..	..	30	..	..	..	..	8	11
$PW \times WO$	263.1	263	263	101	..	..	..	..	..	..	..	14	23	..	..	..	26	12	..	..	..	..	26	26
$PP_6 \times NO$	264	83.1	31.2.3	131	..	..	..	..	..	..	..	..	57	..	..	..	..	..	48	..	..	..	26	20
$NP \times PO$	264.1	264	264	98	..	..	..	11	9	..	..	..	15	..	..	..	..	..	11	..	..	..	52	53
$NN_4 \times WO$	265	31.2.6	188.1	145	..	..	73	51	..	..	..	..	..	..	..	..	..	..	..	..	..	..	21	14
$NW \times NO$	265.1	265	265	41	..	16	..	3	..	..	..	..	..	..	..	..	..	6	..	..	..	..	16	39
$RR \times PO$	266	Stock	83.1.2	141	..	..	..	..	..	..	..	..	..	..	..	40	..	..	..	..	37	..	64	45
$WW \times RO$	268	117.1	44.3	99	..	..	..	..	..	..	..	..	..	..	42	..	..	38	..	..	..	..	19	19
$RW \times WO$	268.1	268	268	26	..	..	..	..	..	..	..	..	..	..	3	1	4	4	..	..	..	..	14	54
$NR_{10} \times PO$	270	254	83.1.2	126	..	..	..	31	..	..	..	..	..	..	..	21	..	..	..	58	..	..	16	13
$PR \times RO$	270.1	270	270	54	..	..	..	..	..	..	..	8	7	..	..	12	..	..	..	..	8	..	19	35
$NR_{10} \times NO$	271	254	31.2.6.2	139	17	..	..	26	..	..	..	..	..	..	..	23	..	..	..	..	..	18	55	40
$NR_9 \times NO$	272	217	31.2.6.2	116	23	..	..	20	..	..	..	..	..	..	..	29	..	..	..	..	..	29	15	13
$NR_9 \times PO$	273	217	83.1.2	75	..	..	..	14	..	..	..	..	..	..	..	16	..	..	..	33	..	..	12	16
$RR_{11} \times WO$	286	44.3	117.1.1	71	..	..	..	..	..	..	..	..	..	32	..	27	..	..	..	..	..	..	12	17
$PP_6 \times WO$	289	83.1	117.1.1	88	..	..	..	..	..	..	38	30	..	..	..	..	..	..	..	..	..	..	20	23
$PW \times PO$	289.1	289	289	53	..	..	..	..	..	9	..	3	..	..	..	..	..	1	..	..	..	..	40	76
$NR \times WO$	290	257	117.1.1	56	..	..	5	3	..	..	..	..	..	..	5	15	..	..	..	..	..	..	28	50
$WW \times RO$	291	117.1.1	257	16	..	..	..	..	..	..	..	..	..	..	4	..	..	1	..	..	..	..	11	69
$RR_{12} \times WO$	298	44.3	117.1.1	64	..	..	..	..	..	..	..	..	..	..	13	18	..	..	..	..	..	..	33	52
$RR_5 \times RO$	308	44.3	44.3.2	99	..	..	..	..	..	..	..	..	22	..	..	27	..	..	..	..	..	..	50	50
$NN \times NO$	313	31.2.6.2	31.2.6.2	57	11	..	..	25	..	..	..	..	..	..	..	..	..	..	..	..	..	..	21	37
$PW \times WO$	314	263	268	112	..	..	..	..	..	..	7	14	..	..	..	..	18	21	..	..	..	..	52	46
$NP \times WO$	316	264	263	129	..	..	8	19	..	..	17	17	..	..	..	..	..	..	..	..	..	..	68	53
$PP \times PO$	320	83.1.3.1	83.1.3.1	57	..	..	..	..	17	..	..	10	..	..	..	..	..	..	..	..	..	..	30	53
$NN \times NO$	321	272	261	15	6	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	7	47
$RW \times WO$	327	268	268	172	..	..	..	..	..	..	..	..	..	..	15	23	21	29	..	..	..	..	84	49
				6524																			2258	

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CYTOLOGICAL STUDIES IN THE GENUS NICOTIANA

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INTRODUCTION

The genus Nicotiana has been a favorite subject for study since the time of KÖLREUTER, his investigation of crosses between *N. paniculata* and *N. rustica* being the first genetical research of which we have a record. Unfortunately, the work of the hybridizers of the eighteenth and nineteenth centuries produced few generalizations of value to genetics, because of the numerous variables under consideration. The foundations of genetics have been laid by experiments where a limited number of genetic differences have been followed and where the fertility of the hybrids was virtually perfect. More recently, however, there has been a trend back to the study of species hybrids, using both the cytological and the pedigree culture methods of technique. The work of SAX on the genus Triticum is an excellent example. Using the general philosophy of genetics built up by intra-specific studies as a basis of his inductions, SAX has been able to throw considerable light , not only upon the genetic behavior of the species composing this genus, but also upon their origin. It was

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thought, therefore, that perhaps a similar series of investigations upon the genus Nicotiana might yield results of some value. There was the further hope that the genus might again be brought to the favorable attention of geneticists. It deserves a high place in their regard, for the plants are easy to grow, crosses are easy to make, large quantities of seed are produced, and the seed retains its viability for several years.

Since the genus is fairly large (about 60 species having been described) it has been impossible for me to make even a relatively complete survey. I have been able to obtain 19 species. In each of these forms, the chromosome number has been determined. In addition, 15 species were selected for hybridization work, and an effort made to cross each of these species with all of the others. In the case of nine hybrids, the cytological behavior of the F_1 plants was investigated.

The species used correspond with the descriptions found in COMES' Monographie du genre Nicotiana (1899), with one or two exceptions. These exceptions are species described since the appearance of this work. Their descriptions can be found in the original publications cited in the bibliography.

METHOD AND TECHNIQUE

The haploid chromosome number in each species was determined both from aceto-carmine preparation made according to Bellings' method and also from permanent slides. A very small part of the bud was cut away at the tip so that a part of each anther could be examined by the aceto-carmine method. The rest was immediately fixed with modified Bouin's solution as developed by ALLEN. Sections were cut 7 to 10 microns thick, and were stained with Haidenhain's iron-hæmatoxylin.

The diploid chromosome numbers of the pure species, and in a few cases of the dwarf species hybrids, were determined by a study of the root tips at a time when the plant, grown in sterile soil, had produced its 6th or 7th leaf. The fixative in this case was strong Flemming's solution, the stain being again Haidenhain's iron-hæmatoxylin.

In the hybridization work, 3 or 4 plants of both species were always used. The parent plants were kept together, but isolated as far as possible from the plants of the other species. The hybridization was made in the greenhouse, and in order to prevent contamination, all buds which could not be used for hybridization were cut off at a very early stage. The flowers were not bagged. Flying insects were excluded from the house by cheesecloth covers; and experiments have shown that with plants having heavy pollen, as is the case with all Nicotiana species, this is a sufficient

precaution. Crosses and reciprocals were made in each case using five buds of every combination. The seeds were always grown in sterilized soil.

THE CHROMOSOMES OF THE VARIOUS NICOTIANA SPECIES

Tabacum section

1. *N. Tabacum* L. Within this species I have made cytological studies of the following varieties: Cuba, *macrophylla*, and a white flowering tobacco. According to COMES, Cuba belongs to the var. *havanensis*. It has lanceolate leaves and pink flowers. *Macrophylla* is a very strong plant with large ovate leaves gradually tapering to a broad clasping base, with rounded but not prominent basal lobes. The flowers are deep red with a stout tube and an abruptly swollen, broad infundibulum; the limb is almost pentagonal, and at the base of every lobe is a whitish, triangular spot. The white tobacco is a small variety bearing a large number of small lanceolate and broadly decurrent leaves tapering towards the base on a relatively short stem. The corolla is relatively small, short, and cream colored.

In each of these three varieties, I found 24 chromosomes in the pollen mother cells during the different stages of the reduction division. The number was very easy to count at the second metaphase or at the first anaphase because the chromosomes at these stages, though small, are clearly separated. Sometimes the first metaphase (figure 19) is exceptionally clear and favorable for the study of chromosome size. At this stage the chromosomes are twice as large as in the second metaphase and lie close together. Figure 19 shows clearly the differences in the chromosome size. The small chromosomes are arranged in the center, the larger chromosomes at the periphery.

There is great difference in the chromosome shape at meiosis and at mitosis. In the cells of the root tips the chromosomes are long and slender, usually curled, and difficult to count. In figure 1 is represented one of the root tip cells of *N. Tabacum* var. *macrophylla* in which it is relatively easy to distinguish 48 chromosomes.

Rustica section

2. *N. rustica* L. In this species I have studied the varieties *humilis*, *brasilia*, and one which is apparently *texana*. The 24 haploid chromosomes of the pollen mother cells, in contrast to those of *Tabacum*, are uniform in size and shape. Their shape is markedly different from that of the *Ta-*

bacum chromosomes, being bean-shaped instead of spherical (figure 18). During the second metaphase they are to be seen clearly because of their separation, but are so small that their shape is vague, and they appear to be spherical.

The somatic chromosomes, though somewhat larger than those of *Tabacum*, are entwined and difficult to distinguish. It was impossible to make counts on preparations of root tips from the small *humilis* variety because the cells are very small and the chromosmes much entwined. Only in *brasilia* was I able to find clear plates (figure 2) where the chromosomes are manifestly 48.

3. *N. paniculata* L. The material used had all of the characteristics of true *N. paniculata*—a long, slender corolla tube with short, symmetrical, greenish corolla lobes. The upper leaves were lanceolate, the basal leaves were ovate, slightly cordate, and with long petioles.

The 12 chromosomes counted during different stages of the reduction division in the pollen mother cells (figure 25) seem to be identical in shape with those of *N. rustica*. In the cells of the root tips the chromosmes are very slender, long, and twisted; but 24 are easily distinguished (figure 8).

4. *N. glutinosa* L. The material used in this study was a small branched plant with small, cordate, long petioled, extremely glandular leaves. The flowers were short and cylindrical below, but suddenly swollen above into an irregular, obliquely one-sided funnel. The limb was bilabiate, the stigma and anthers being connivent just under the middle lobe of the upper lip. The color was light yellow tinged with red.

Twelve chromosomes were counted in the different stages of the pollen mother cells. The chromosomes are uniform in size and shape, the spherical shape giving them a greater resemblance to those of *N. Tabacum* than to the bean-shaped chromosomes of *paniculata* or *rustica*. They are only one-half as large as those of *paniculata*. During the first metaphase they lie very close together (figure 26), but are very clear and distinct in the first anaphase. The differences between the chromosomes of *glutinosa* and *paniculata* are visible also in the somatic cells, those of the former (figure 6) being shorter and slenderer than the latter.

5. *N. glauca* Grah. This species is a tree tobacco with long, petioled, glaucous leaves. The flowers are pale yellow with tubes similar to those of *paniculata*. The stem and leaves are bluish green. The chromosome number was found to be 12 in the pollen mother cells (figure 23) and 24 in the root tips (figure 9). The somatic chromosomes are extremely large, long, and entwined, but because of the large cells and nuclei they are well scattered and therefore are easily distinguished.

6. *N. tomentosa* R. et. P. The two year old plants are not yet in flower, but their general character shows them to be true *N. tomentosa* (*Lehmannia tomentosa* Spr.). The chromosome number was counted in the cells of the root tips and found to be 24 (figure 10).

Petunioides section

7. *N. Bigelovii* Wats. According to SETCHELL (1912) different varieties of *N. Bigelovii* exist. The plants used were small, their first leaves being slightly decurrent, nearly petioled, in fact, the later leaves becoming characteristically truncate, auriculate, and partly clasping at the base. The first leaves were more or less elongate deltoid, the later leaves more lanceolate. The corolla tube was narrow, and the corolla limb deeply divided into long pointed lobes. The corolla was pure white. Chromosome counts were made most easily during the first and second metaphases. During the first metaphase (figure 16) the chromosomes appear as bean-shaped objects about equal in size but not quite so large as those of *N. rustica* and *N. Tabacum*. In the root tips 48 chromosomes were counted (figure 3).

8. *N. viscosa* Lehm. (?). This species was raised from seeds obtained from a Gray Herbarium sheet labeled *N. viscosa*. At the same time I obtained seeds from another Gray Herbarium sheet labeled *N. attenuata*. Both groups of plants had the same morphological characters. Both lots may be *N. attenuata*, but I have labeled them *N. viscosa* (?) because I raised other specimens received from Dr. T. H. GOODSPEED as *N. attenuata* which differed somewhat from the first two lots. The latter group of plants was rather similar to *N. acuminata*, but very much smaller. These specimens, in fact, seemed to be much reduced acuminatas, and therefore correspond more closely to the taxonomic description of *N. atenuata*. I am not certain of the identification, however, for these species are quite similar to each other, and perhaps should be grouped together.

The pollen mother cells are nearly as large as those in *macrophylla*. The chromosomes also are quite large, lying close together during the first metaphase (figure 20) and more distant during the second. During the first and second metaphases as during the first anaphase I counted 24 chromosomes.

9. *N. nudicaulis* Wats. *N. nudicaulis* is a very small plant with dark green ovate leaves narrowly decurrent. The corolla is shorter and broader than in *N. trigonophylla*. The tube is somewhat brownish, the limb cream. The corolla lobes overlap.

The chromosomes of the pollen mother cells are not easy to count at any stage of the reduction division, being small and usually rather dumb-

bell shaped. In the very early first metaphase the chromosomes are somewhat more spherical, however, and 24 could be distinguished (figure 21). In the root tips, it is relatively easy to count 48 chromosomes, the chromosomes being slender and well separated (figure 4).

10. *N. suaveolens* Lehm. The plants used were branched, with the lower leaves ovate, sessile, clasping at the base and pointed at the tip. The upper leaves were sharply lanceolate and clasping. The corollas were ivory white, salver shaped and with long slender, non-infundibular tubes. The corolla limb is large with broad, shallow rounded lobes.

During the first metaphase of the pollen mother cells, the 16 chromosomes always lie close together (figure 22). They are most easily counted in the first anaphase or in the second metaphase. Cells were found with both plates of a first anaphase lying in the same section in such a manner that by focussing 16 chromosomes can be counted in each. In the first anaphase, the chromosomes are seen as curved rods or as spheres depending on their position. In both the first and the second metaphases they are more or less spherical. During the second metaphase they are more separated and are easy to count. The chromosomes in this species are relatively very small though the cells in comparison with those of the other species are very large. In the cells of the root tips 32 chromosomes could be counted. The chromosomes here are rather slender, comparatively short, and often U-shaped (figure 5).

11. *N. sylvestris* Speg. *et* Comes. In general habit as in leaf shape this species of the Petunioides section is very similar to certain *Tabacum* varieties. Its corolla is white, and very long and slender, the tube being inflated in the middle. The corolla limb consists of 5 broadly triangular lobes. The flowers ordinarily droop.

In the pollen mother cells during the heterotypic metaphase 12 spherical chromosomes, closely arranged at the plate, were counted (figure 24). The first anaphase and the second metaphase are the best for making counts, the 12 chromosomes then lying fairly distant from each other. In the cells of the root tip preparations the chromosomes are long, U-shaped, quite large, and spread out, so that it is easy to distinguish 24 chromosomes (figure 7).

12. *N. acuminata* Grah. This is a branched plant with petioled leaves, cordate below and lanceolate above. The coarse, spine-like glandular hairs on the surface and on the margin are characteristic. The corolla is white with a cylindrical tube and shallow, rounded lobes on the limb.

Twelve chromosomes were counted during the different stages of the reduction division of the pollen mother cells (figure 27). It is easier to

count them at the second metaphase or the first anaphase. The chromosomes after the second division are long and U-shaped, somewhat similar to those of the somatic cells, though during the other stages they are almost spherical. In some other plants this is a common phenomenon; but in the rest of the Nicotiana species it apparently does not occur, the chromosomes after the second division ordinarily resembling those of the earlier stages.

13. *N. trigonophylla* Dun. This is a very small plant with broadly lanceolate leaves, narrow at the base and then expanded into broad, partly clasping auricles. The corolla is very small with a short, cylindrical tube. The corolla limb consists of five broad, obtuse, and shallow lobes. The flowers are a deep cream tinged with green.

In all stages of the reduction division in the pollen mother cells 12 chromosomes were found (figure 28). The chromosomes were all of about the same size, spherical, and distinctly spread, especially during the second metaphase. In the somatic cells of the root tip they are easily distinguished as 24 relatively straight and slender chromosomes (figure 11).

Seeds from a specimen at the Gray Herbarium were obtained under the name of *N. Palmeri* A. Gray. Plants raised from them exhibited no particular differences from *N. trigonophylla*, except that the corolla was lighter and the leaves more lanceolate than those of my *trigonophylla* plants. Twelve chromosomes were counted in the different stages of the pollen mother cells (figure 29). Permanent slides of the young root tips show 24 nicely spaced chromosomes (figure 12).

14. *N. longiflora* Cav. This is quite a tall plant with broad, lanceolate or oblanceolate leaves, coarsely bullate and rugose above, the epidermis smooth except for coarse, spine-like, glandular hairs on the surface and margins. It early develops a characteristically compact rosette of large, coarse leaves which lie flat on the ground, the rosette persisting for a considerable time before the flowering stalk develops. The corolla tube is extremely long and slender. The broad, spreading limb is deeply divided into five moderately broad, blunt-pointed lobes. During the first metaphase (figure 30) and the first anaphase I counted 10 chromosomes.

15. From *N. plumbaginifolia* Viv., a species closely related to *N. longiflora*, I have obtained good preparations of the root tips in which 20 well separated chromosomes of different shapes and sizes could be clearly distinguished (figure 13).

16. *N. alata* Lk. et Otto. The plants which I have investigated came from commercial seed, and showed great variability. Concerning their origin I know nothing. They may have been segregates from

crosses between *N. alata* and *N. Forgetiana*, since it is supposed that the different colored varieties of *N. Sanderae* have originated by crossing *N. alata* with *N. Forgetiana* (HEMSLEY 1905). Some of these plants, however, seem to be the pure *alata* type. I have investigated the pollen mother cells of about 25 plants differing in habit, shape and corolla color, making both acetocarmine preparations and permanent slides. In some plants I found 8 chromosomes in all of the stages. In other plants I obtained irregular results. It was doubtful whether the number was 8 or 9, because one of the chromosomal masses could be considered as two small chromosomes lying close together, or as end portions of a single chromosome. Since I found the somatic chromosomes to be very different in shape and size, I am inclined to accept the second hypothesis. In the cells of the root tips I could distinguish 16 chromosomes; one pair very long and S-shaped, one pair long and straight, 4 pairs U-shaped and of different sizes, one pair very short and rod-shaped, and a small pair almost spherical (figure 14). Since the difference in size is so extreme in the somatic cells, the relative size proportions ought not to disappear in the pollen mother cells even though the contraction of the chromosomes there is very great. Some spindles of the first metaphase show clearly that the chromosomes are mostly long and dumb-bell-shaped, though in polar view they appear almost spherical. I am inclined to believe, therefore, that the position of some of the 4 U-shaped chromosomes may be such as to present only the tips which could appear as separate chromosomes lying close together.

It may be possible, also, that this occurrence is due to an earlier split of some of the chromosomes as described by WINGE (1917) for *Chelidonium majus* and *C. majus* var. *laciniatum*. WINGE explained BÖNICKE's determination of the chromosome number in *Chelidonium majus* as eight instead of six by the observation that the chromosomes in the pollen mother cells of this species have a marked power of precocious division. He found that "in the early anaphase, the six chromosomes moving towards the pole are not only divided into two, but into four, thus resembling a *Sarcina*, and, indeed, it would seem as if still finer subdivisions were present, which renders it difficult to ascertain the true number of chromosomes." The splitting phenomenon he found to be still more frequent in var. *laciniatun* than in the typical *Chelidonium majus*, and concluded: "The fact is of considerable interest from a theoretical point of view, as the distinct division in the heterotypic anaphase shows that the chromosome must not be regarded as an indivisible unit, but as a complexity, in which an inner differentiation or duality is already present. And in all probability,

it is also actually existent even when no outward and visible sign of division is discernible."

From the many plants in which I have studied chromosome number in the pollen mother cells I shall present some figures drawn from temporary slides made by the acetocarmine method. By this method I was able to distinguish the rod shape of the chromosomes not only in side view on the spindles, but also in polar view, for the acetocarmine fluid included between the slide and the cover glass allowed the chromosomes to move slightly and to take different positions during the observation. In cases in which questionable numbers were found, the final conclusion was drawn only after exact observations of the questionable chromosomes in different positions. In figure 34, one plate of the second metaphase was in polar view, and appeared to have 9 chromosomes; the other was not exactly in polar view, and only 7 chromosomes could be counted with certainty. After standing, however, the position of the material was changed, the plate with the 7 chromosomes appearing as a regular spindle in which the chromosomes were more or less indistinguishable, and the other plate showing clearly 8 chromosomes.

In all of the other cells counted the number of the chromosomes was always 8 (as in figure 35). Figure 36 shows the shape of the chromosomes of the first metaphase in side view. In figure 32 all chromosomes clearly show splitting, and are similar in shape to those in figure 21*b*, *d*, and *h* presented by WINGE for *Chelidonium majus*. Figure 33 is very similar to figure 21*i* of WINGE. The chromosomes can be counted as 10, but since in two places chromosomes lie so close together, I am inclined to believe that they are two long chromosomes instead of four.

GOODSPEED in a preliminary note (1923) has reported for different Nicotiana species the haploid numbers 9, 12, and 24, but for some of them he makes the following reservation.

> Three of these counts are open to some question. In the case of *N. alata*, homotypic anaphase plates in the fixed material show 10 chromosomes in some cases, although the predominating number in such stages is 9. Similarly, *N. longiflora* can be counted as 9 or 10, but in this case the predominating number is 10. Only a small amount of fixed material of *N. suaveolens* was available, and there is some doubt as to whether the number is not larger than 12, possibly 18.

Later, (1924) he describes a very interesting case of *N. alata* in which, after the second division, two of the anaphase plates have 8, and the other plates 10 chromosomes. Failure of conjugation in one chromosome pair, he believes, will account for this condition, because in other cases he found

9 chromosomes in three nuclei of the tetrads. For *N. suaveolens*, he decides that the number is 18. At the IVth International Congress of Botany at Ithaca, I showed Dr. GOODSPEED on one of my slides a single section in which both the plates of the homeotypic anaphase contained only 16 chromosomes. In a recent publication (GOODSPEED and CLAUSEN 1927), this number is admitted to be the correct one.[1]

17. *N. Langsdorffii* Weinm. This is a small plant with sessile, elliptical-lanceolate leaves, narrowed and decurrent at the base. The corolla is funnel-shaped below with a gibbous ring above and a concave, spreading limb slightly notched into five broad, shallow lobes, greenish yellow and pendent.

At the various stages, 8 chromosomes were observed (figure 31), though it is not always easy to count them for reasons similar to those discussed in the case of *N. alata*. In the root tips (figure 15), I found 16 chromosomes similar in shape to the chromosomes found in the root tips of *alata* (figure 14).

Polydiclia section

18. *N. quadrivalvis* Pursh. This is a small plant somewhat similar to *Bigelovii*, except that the leaves are not elongate deltoid but broadly deltoid. The corolla is shaped somewhat similarly to that of *Bigelovii*, but is much larger. The capsule is spherical and twice as large as that of *Bigelovii*. During the various stages of the reduction division in the pollen mother cells, 24 chromosomes were easily counted (figure 17).

[1] Since this paper was finished, Miss RUTTLE has published an interesting study of the chromosomes in *N. alata* var. *grandiflora* (Univ. of California Pub. Bot. **11**: 159–176, 1927). She has gone about her investigation in a very workmanlike manner, making extensive counts and even endeavoring to identify the individual chromosomes in various plates. She concludes that there are 18 chromosomes in the somatic cells, the usual distribution being 9–9, though 8–10 distributions occur in about nine percent of the cases. She believes that in my previous work (Jahrb. d. Univ. Sofia, Agric. Fac. **3**: 37–86, 1925) where a diploid number 16 was reported for the species, I incorrectly figured two chromosomes as one—a very long one. It is possible that Miss RUTTLE'S view is correct. But I must call attention to two points which should be weighed carefully before a decision is made. First, Miss RUTTLE figures 3 pairs of U-shaped chromosomes in her Figure 1a plus 2 pairs the ends of which lie very close together (upper center). This latter configuration I believe to be a single pair of chromosomes. Second, and this is my critical point, in certain hybrids with species having 10 chromosomes as the haploid number, the reduction division of the pollen mother cells shows the number of univalents properly to be expected if the chromosome number for *N. alata* is $x=8$. For example, in heterotypic spindles of *N. longiflora*×*Sanderae*, *N. longiflora*×*alata*, *N. plumbaginifolia*×*alata*, *N. plumbaginifolia*×*Langsdorffii*, *up to two lagging univalent chromosomes* occur and are often to be seen approaching the poles, whereas the bivalents are still at the equator (see figure 37); and *regularly more than two lagging chromosomes* appear during the homeotypic division.

19. *N. multivalvis* Lindl. This is a plant very similar to *quadrivalvis*, only larger, with very large corolla and capsules. Counts were made only from temporary slides with actetocarmine staining and 24 chromosomes were counted in the pollen mother cells.

HYBRIDIZATION RESULTS

In the hybridization experiments the following species of Nicotiana were used:

1. *N. alata*
2. *N. Sanderae*
3. *N. longiflora*
4. *N. plumbaginifolia*
5. *N. acuminata*
6. *N. trigonophylla*
7. *N. sylvestris*
8. *N. suaveolens*
9. *N. Bigelovii*
10. *N. nudicaulis*
11. *N. Langsdorffii*
12. *N. paniculata*
13. *N. glutinosa*
14. *N. rustica*
15. *N. Tabacum macrophylla*

The technique of hybridization has already been described. Crosses were made between nearly all of these species in both directions, pollinating five (or more) flowers in each case. The few exceptions were the omission of *N. Sanderae* as female, of nine combinations with *N. nudicaulis*, and of one combination with *N. rustica*. The results obtained are recorded in table 1.

Two weeks after the capsules were harvested the seeds were sown. For every cross several sowings were made according to the quantity of seed harvested. If all of the flowers which were pollinated produced capsules, 100 seeds were first taken for the germination test, the rest being sown in sterilized soil for the production of F_1 plants. For crosses from which one or two capsules only were harvested, or in which only a few seeds developed, the material was used in the germination experiment. If germination occurred, the seedlings were transferred to pots of sterilized soil. If no germination occurred after three weeks, successive sowings were made until all the seed was used. The germination experiment was made in the usual way on blotting paper, the papers being kept in an incubator at a temperature of 27° to 30° C. Sometimes it happened that not a single seed germinated in the soil while a few germinated in the germination test; but it also happened that a few germinated in the soil though none appeared in the germination test.

A prospectus of all the crosses is given in table 1. In the horizontal rows are given the species used as female, and in the vertical row those used as male. The chromosome number for each of the species follows the name. The crosses in which the flowers have dropped after pollination are de-

noted by *o*, those in which the harvested capsules contained no seeds or non-germinative seeds by *d*, those which produced defective seedlings by *s*, those in which mature hybrids were produced by *h*.

TABLE 1.

Results of the hybridization experiments.

SPECIES USED AS MALES		SPECIES USED AS FEMALES														
		N. alata	*N. Sanderae*	*N. Langsdorffii*	*N. longiflora*	*N. plumbaginifolia*	*N. acuminata*	*N. sylvestris*	*N. trigonophylla*	*N. paniculata*	*N. glutinosa*	*N. suaveolens*	*N. Bigelovii*	*N. nudicaulis*	*N. rustica*	*N. Tabacum*
		X=8	8	8	10	10	12	12	12	12	12	16	24	24	24	24
N. alata	X=8	..	..	h	h	h	d	d	d	s	o	s	d	d	..	h
N. Sanderae	8	h	..	h	o	o	o	d	d	h	d	o	d	..	h	h
N. Langsdorffii	8	h	..	..	o	h	o	o	s	h	s	o	s	d	h	s
N. longiflora	10	o	..	o	..	h	d	o	d	s	d	h	s	..	d	s
N. plumbaginifolia	10	o	..	o	h	..	d	o	d	s	d	h	s	..	d	s
N. acuminata	12	o	..	o	o	o	..	o	o	o	d	d	d	..	d	o
N. sylvestris	12	o	..	o	o	o	o	..	o	d	h	o	s	o	o	h
N. trigonophylla	12	o	.	o	o	o	o	o	..	d	o	o	o	h	d	o
N. paniculata	12	o	..	o	o	s	o	o	d	..	d	o	s	o	h	o
N glutinosa	12	o	..	o	o	o	o	o	d	h	..	h	h	..	d	h
N. suaveolens	16	o	..	o	o	o	o	o	d	s	d	..	h	d	s	d
N. Bigelovii	24	o	..	o	o	o	o	o	d	d	h	h	..	..	d	d
N. nudicaulis	24	o	..	o	..	..	o	..	d	d	h	o	h	..	d	d
N. rustica	24	o	..	o	o	o	o	o	d	d	o	o	o	d	..	o
N. Tabacum	24	o	..	o	o	o	d	h	d	d	d	s	h	s	h	..

Combinations marked .. were not made
Crosses marked o produced no capsules
Crosses marked d produced capsules without viable seeds
Crosses marked s produced defective seedlings
Crosses marked h produced mature hybrids

Thus *N. alata*, used as female, gave hybrids with *Langsdorffii* and *Sanderae*; but when pollinated by other species it dropped its flowers. When used as male, however, *alata* produced hybrids not only with *Langsdorffii*, but also with *longiflora*, *plumbaginifolia*, and *Tabacum*. *Langsdorffii* as female shows the same results as *alata*, but as male exhibits some differences.

Trigonophylla, which is placed by DUNAL (1852) in the *Rustica* and by COMES in the Petunioides section, produced defective seedlings when pollinated by *Langsdorffii* of the Rustica section, and with *alata* and *Sanderae* of the Petunioides section it did not even give viable seeds. *Glutinosa*

which is in the Rustica section, gave the same results when pollinated with these species. In general, the species with 12 haploid chromosomes show a very weak power of hybridization, *acuminata* and *trigonophylla* producing no hybrids with any of the species used in these experiments.

In species with 24 chromosomes the hybridizing ability is somewhat greater than in those with lower chromosome numbers, but compatibility between species shows no significant relation to taxonomic status.

Out of 186 crosses 84 were made reciprocally, and 67 of these crosses were between species differing in their chromosome number. In 56 of the latter no mature hybrids were obtained when the species with the lower chromosome number was used as female, but a higher degree of compatibility was shown when the species with the higher chromosome number was used as female. In 8 crosses where the species with the lower chromosome number was used as female the pollinated flowers dropped, while from the reciprocals mature hybrids were raised. In 4 other crosses where the species with the lower chromosome number was used as female, defective seeds were produced; while in the reciprocals mature hybrids were obtained. In 13 crosses the pollinated flowers dropped from the species with the lower chromosome number, while the reciprocals produced defective seedlings. In 19 crosses the pollinated flowers of the species with the lower chromosome number also dropped, although the reciprocals produced defective seeds. In one cross both types of crossing resulted in defective seedlings, and in another only defective seeds were produced. In 9 crosses the incompatibility between the species was so great that the pollinated flowers dropped in both the direct and reciprocal crosses. In one cross defective seeds were produced by using the species with the smaller number of chromosomes as female, and defective seedlings by the reciprocal.

In 8 reciprocal crosses the degree of compatibility appeared to be greater when the species with the lower chromosome number was used as female, but none of these crosses produced mature hybrids. In 6 of these crosses the flowers of the species with the lower chromosome number produced defective seeds, while the flowers of the reciprocals dropped after pollination. In 2 crosses defective seedlings were produced when the species with the lower chromosome number was used as female; in one of the reciprocals defective seeds were produced, in the other the flowers dropped.

Mature hybrids were obtained in both directions from only three of the 67 crosses involving species differing in their chromosome number, but in two of them (*glutinosa*×*Bigelovii* and *sylvestris*×*Tabacum*) a much

larger number of plants per capsule were obtained when the female parent had the higher chromosome number.

Mature hybrids were obtained in both directions from only two (*alata*×*Langsdorffii* and *longiflora*×*plumbaginifolia*) of the 17 reciprocal crosses between species having the same chromosome number. Of 10 reciprocally made crosses between species with 12 haploid chromosomes, 4 did not produce seeds, no matter which parent was used as female. In 3 crosses the flowers dropped after pollination, while their reciprocals produced defective seeds. In 1 cross defective seeds were produced by both parents. From 2 crosses (*glutinosa*×*sylvestris* and *paniculata*×*glutinsoa*) a few very weak mature hybrids were raised, although their reciprocals produced respectively defective seeds and nothing.

Out of 5 reciprocally made crosses between species with 24 haploid chromosomes, 2 produced very vigorous hybrids, while their reciprocals produced defective seedlings and nothing. In a third cross defective seedlings were produced in one direction and defective seeds in the other. In the fourth cross both female parents produced defective seeds. In the last cross the seeds were defective in one female parent and the flowers dropped in the other.

DESCRIPTION OF THE SPECIES HYBRIDS

A. Species hybrids which did not reach maturity.

Of the various crosses made, 19 produced hybrids which died in early seedling stages.

a. Hybrids obtained by crossing species having 12 with species having 8 as the haploid chromosome numbers are:
   1. *N. trigonophylla*×*Langsdorffii*
   2. *N. glutinosa*×*Langsdorffii*
   3. *N. paniculata*×*alata.*

b. Hybrids obtained by crossing species having 12 and 10 as the haploid chromosome numbers are:
   4. *N. paniculata*×*longiflora*
   5. *N. paniculata*×*plumbaginifolia*
   6. *N. plumbaginifolia*×*paniculata.*

c. Hybrids obtained by crossing species having 16 and 8 as the haploid chromosome numbers are:
   7. *N. suaveolens*×*alata.*

d. Hybrids obtained by crossing species having 12 and 16 as the haploid chromosome numbers are:
   8. *N. paniculata*×*suaveolens.*

e. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers are:
   9. *N. Bigelovii* × *Langsdorffii*
   10. *N. Tabacum* var. *macrophylla* × *Langsdorffii.*
f. Hybrids obtained by crossing species having 24 and 10 as the haploid chromosome numbers are:
   11. *N. Bigelovii* × *longiflora*
   12. *N. Bigelovii* × *plumbaginifolia*
   13. *N. Tabacum* var. *macrophylla* × *longiflora*
   14. *N. Tabacum* var. *macrophylla* × *plumbaginifolia.*
g. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers are:
   15. *N. Bigelovii* × *sylvestris*
   16. *N. Bigelovii* × *paniculata.*
h. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers are:
   17. *N. rustica* × *suaveolens*
   18. *N. suaveolens* × *Tabacum* var. *macrophylla.*
i. Hybrids obtained by crossing species having 24 and 24 as the haploid chromosome numbers are:
   19. *N. nudicaulis* × *Tabacum* var. *macrophylla.*

Of these crosses, only *N. suaveolens* × *Tabacum* var. *macrophylla* (germination 82 percent), *N. Tabacum* var. *macrophylla* × *plumbaginifolia* (germination 3 percent), and *N. nudicaulis* × *Tabacum* var. *macrophylla* (germination 16 percent) yielded a great number of seedlings. In all other crosses the germination was very low. In the cross *N. Tabacum* var. *macrophylla* × *plumbaginifolia*, the seedlings all died after developing the fourth or fifth true leaf. In most of the crosses the seedlings died before developing the second true leaf.

B. Species hybrids which reached maturity.
   a. Hybrids obtained by crossing species having 8 as the haploid chromosome number.

I. *N. alata* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 81 percent. The ten hybrids raised were sterile. They were vigorous but variable, which may indicate that *Sanderae* is heterozygous for various genes, since in other species crosses where *Sanderae* was used the hybrids were also variable.

Nearly all of the hybrids resembled *N. Sanderae* in habit of growth. The leaves were an intermixture of the characteristics of both parents. In corolla color the *Sanderae* parent was dominant; but in size and shape the flowers were nearly intermediate.

II. *N. alata* × *Langsdorffii*

The germination of the seeds obtained was 80 percent (93 percent in the reciprocal). The ten hybrids raised were fertile, vigorous, uniform, and in habit of growth resembled *Langsdorffii*. The leaves were nearly intermediate in color, size and shape. The flowers also were intermediate in color, size and shape. The pollen-grains were intermediate in color—slightly bluish.

III. *N. Langsdorffii* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 62 percent. The ten hybrids raised were fertile, vigorous, and variable in habit of growth, though resembling *Langsdorffii*. The leaves were different in size and shape in the different individuals. In respect to corolla color, *Sanderae* was dominant. In size and shape the flowers were different in the different individuals. The pollen-grains were intermediate in color—light bluish.

b. Hybrids obtained by crossing species having 10 and 8 as the haploid chromosome numbers.

IV. *N. longiflora* × *alata*

The germination of the seeds obtained was 95 percent. The fifty hybrids raised were sterile. They developed slowly into vigorous, uniform plants, in habit of growth an intermixture of the characteristics of both parents. The leaves were slightly rugose, like those of *longiflora*. The flowers, which remained slightly closed during the day, were intermediate in size and shape, though with the corolla lobes bluish externally as in *longiflora*.

V. *N. longiflora* × *Sanderae*

I was unsuccessful in this cross; but ten hybrids were raised from seeds obtained by DR. A. J. MANGELSDORF. The plants were sterile; and though variable in size and in habit of growth, were quite vigorous. The leaves of each individual were slightly rugose like those of *longiflora*. The flowers, although somewhat variable, resembled the two parents in size and shape. They remained slightly closed during the day like those of *longiflora*.

VI. *N. plumbaginifolia* × *alata*

The germination of the seeds obtained was 49 percent. The fifty hybrids raised were sterile. They developed slowly to vigorous, uniform plants, in habit of growth an intermixture of the traits of both parents. The leaves were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, bluish externally like *plumbaginifolia*, and remained slightly closed during the day.

VII. *N. plumbaginifolia* × *Langsdorffii*

The germination of the seeds obtained was 35 percent. The thirty hybrids raised were sterile. They were vigorous, uniform, and in habit of growth intermediate between the parents. The leaves resembled *Langsdorffii* in color, but were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, shape and color. The corolla lobes were yellowish on the upper side, but slightly bluish underneath. The pollen-grains were intermediate in color—slightly bluish.

c. Hybrids obtained by crossing species both having 10 as the haploid chromosome number.

VIII. *N. longiflora* × *plumbaginifolia*

The germination of the seeds obtained was 96 percent (81 percent in the reciprocal). The ten hybrids raised were fertile, very vigorous, uniform, and in habit of growth intermediate between the parents. The leaves and the flowers were also intermediate in their characteristics.

d. Hybrids obtained by crossing species having 12 and 8 as the haploid chromosome numbers.

IX. *N. paniculata* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 28 percent. The ten hybrids raised were sterile. They varied markedly in vigor, from very weak to very vigorous; they also varied in habit of growth. The leaves were variable in the different individuals, but were nearly all petiolate as in *paniculata*. The flowers in all were as red as those of the *Sanderae* parent, varying in size but more closely resembling *paniculata* in shape.

X. *N. paniculata* × *Langsdorffii*

The germination of the seeds obtained was 12 percent. The ten hybrids raised were sterile. The plants were uniformly very weak, in habit of growth resembling *Langsdorffii*. The petiolate leaves resembled *paniculata*

rather closely. The flowers in size and shape resembled *Langsdorffii*. The pollen-grains were intermediate in color—slightly bluish.

e. Hybrids obtained by crossing species both having 12 as the haploid chromosome number.

XI. *N. paniculata*×*glutinosa*

The germination of the seeds obtained was less than 1 percent. The five hybrids raised were sterile. The plants were very weak at the beginning, but quite vigorous at the end. They exhibited little variablity, and were an intermixture of the characteristics of both parents. The first leaves were oblong, the later ones cordate, thus resembling *glutinosa*. The flowers were yellow, tinged with pink, and were intermediate in size and shape.

XII. *N. glutinosa*×*sylvestris*

The germination of the seeds obtained was 5 percent. The two hybrids raised were sterile. They developed slowly and were very weak, though uniform and in habit of growth intermediate between the parents. The leaves were nearly petiolate as those of *glutinosa*, but oblong like those of *sylvestris*. The flowers were pink as in *glutinosa*, but in size and shape intermediate.

f. Hybrids obtained by crossing species having 16 and 10 as the haploid chromosome numbers.

XIII. *N. suaveolens*×*longiflora*

The germination of the seeds obtained was 22 percent. The thirty hybrids raised, though sterile, were very vigorous and in habit of growth resembled *suaveolens*. The leaves also resembled *suaveolens*. The flowers were more like *suaveolens* in size and shape; but in corolla color *longiflora* was dominant, the corolla lobes being bluish underneath.

XIV. *N. suaveolens*×*plumbaginifolia*

The germination of the seeds obtained was 2 percent. The fifteen hybrids raised, though sterile, were very vigorous, uniform, and in habit of growth resembled *suaveolens*. The leaves and flowers resembled *suaveolens*, though in corolla color *plumbaginifolia* was dominant, the corolla lobes being bluish underneath.

g. Hybrids obtained by crossing species having 16 and 12 as the haploid chromosome numbers.

XV. *N. suaveolens*×*glutinosa*

The germination of the seeds obtained was 88 percent. The five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *suaveolens*. In size and shape the leaves were intermediate, but petiolate as those of *glutinosa*. The flowers were pink like those of *glutinosa*, but in size and shape resembled *suaveolens*.

h. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers.

XVI. *N. Tabacum, Cuba*×*alata*

The germination of the seeds obtained was less than 1 percent. The two similar hybrids raised were sterile, very weak, and in habit of growth resembled *Tabacum*. The characteristics of both leaves and flowers were more nearly like those of *Tabacum*.

XVII. *N. Tabacum*, white tobacco×*Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. Three hybrids were raised from a cross in which *Sanderae* Cl_{10} was used. All of them were sterile, quite weak, and though different, were more nearly like *Tabacum*. The leaves and the flowers resembled *Tabacum* in size and shape, but the corolla color of *Sanderae* was dominant. From another cross, where *Sanderae* N16 with bluish corolla was used, the four hybrids obtained showed the result of segregation in corolla color, three being bluish and one white. These hybrids also were sterile, weak, and variable in habit, though resembling *Tabacum*.

XVIIII. *N. rustica*×*Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. The four hybrids raised were sterile. The plants varied markedly in vigor and size, although in habit of growth they resembled *rustica*. The leaves of all were petiolate, and in size and shape were like those of *rustica*. The flowers although different in size and shape in the different individuals, resembled *rustica*, except that the red corolla color of *Sanderae* was dominant.

XIX. *N. rustica*×*Langsdorffii*

The germination of the seeds obtained was less than 1 percent. The only hybrid raised was sterile, very weak and in habit of growth like *rustica*. The leaves were petiolate, resembling *rustica*, in size and shape. The

flowers also resembled *rustica* in size and shape. The pollen grains were intermediate in color—slightly bluish.

i. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers.

XX. *N. nudicaulis*×*trigonophylla*

The germination of the seeds obtained was 46 percent. The twenty hybrids raised were sterile, though quite vigorous, uniform and with a habit of growth like *nudicaulis*. The leaves were also more like those of *nudicaulis*. The corolla limb was curved as in *nudicaulis*, but the tube intermediate between those of the parents in length.

XXI. *N. glutinosa*×*nudicaulis*

The germination of the seeds obtained was 10 percent. The twenty-eight hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the characteristics of both parents. In color, the leaves were like those of *nudicaulis*, but petiolate as in *glutinosa*. The flowers were like those of *glutinosa* in color and shape, and like those of *nudicaulis* in size.

XXII. *N. Bigelovii*×*glutinosa*

The germination of the seeds obtained was 98 percent, but less than 1 percent in the reciprocal. The fifty hybrids raised were sterile, vigorous, uniform and in habit of growth resembling *Bigelovii*. The leaves were petiolate like those of *glutinosa*, but in size, shape and color resembled *Bigelovii*. The flowers were slightly pink, but in size and shape resembled *Bigelovii*.

XXIII. *N. Tabacum* var. *macrophylla*×*sylvestris*

The germination of the seeds obtained was 50 percent. The seventy-five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Tabacum*. The leaves and the flowers resembled *Tabacum*, but the corolla tube was more slender, the corolla limb smaller and the corolla lobes narrower than those of *Tabacum*. The reciprocal cross was made by using another *Tabacum* variety as the male parent. Here the germination of the seeds obtained was less than 1 percent. The three hybrids raised behaved in respect to the parental characters as in the cross *macrophylla*×*sylvestris*.

XXIV. *N. Tabacum* var. *macrophylla*×*glutinosa*

The germination of the seeds obtained was 25 percent. The twelve hybrids raised were sterile, very weak, variable in size and habit, an intermixture of the traits of both parents. The leaves were petiolate, small and ovate, more like those of *glutinosa*. The flowers were as red as those of *macrophylla*, but in size and shape were intermediate.

XXV. *N. rustica*×*paniculata*

The germination of the seeds obtained was 35 percent. The fifteen hybrids raised were partially fertile, quite vigorous, uniform and in habit of growth resembled *rustica*. The leaves and the flowers approached closely to those of *rustica*, but the corolla tube was somewhat intermediate in length.

j. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers.

XXVI. *N. Bigelovii*×*suaveolens*

The germination of the seeds obtained was 84 percent (98 percent in the reciprocal). The thirty-three hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Bigelovii*. The leaves and flowers were more like those found in *Bigelovii*.

k. Hybrids obtained by crossing species having 24 as the haploid chromosome number.

XXVII. *N. Bigelovii*×*nudicaulis*

The germination of the seeds obtained was 15 percent. The twenty hybrids raised were sterile, vigorous, uniform and in habit of growth an intermixture of the traits of both parents. The leaves resembled *Bigelovii*. The flowers were intermediate in color, but were small with curved corolla as in *nudicaulis*.

XXVIII. *N. Bigelovii*×*N. Tabacum* var. *macrophylla*

The germination of the seeds obtained was 15 percent. The twenty-five hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the traits of both parents. The leaves and flowers were also intermediate.

XXIX. *N. rustica*×*N. Tabacum* white tobacco

The germination of the seeds obtained was less than 1 percent. The two hybrids raised were slightly fertile, vigorous, and in habit of growth inter-

mediate. The leaves were petiolate, and in shape were more nearly like *rustica*; but were intermediate for size. The flowers were intermediate in color, size and shape.

CYTOLOGICAL OBSERVATIONS ON CERTAIN NICOTIANA SPECIES HYBRIDS

A. *N. alata*×*Langsdorffii*

The heterotypic and homeotypic divisions in the pollen mother cells are normal.

B. *N. longiflora*×*Sanderae*

The reduction division shows a picture very similar to that of the Drosera hybrids (ROSENBERG 1909). There are eight bivalents and two univalents. Sometimes it appears that in an earlier stage of the heterotypic metaphase the univalents are in the same plane as the bivalents, but frequently one observes spindles with two lagging chromosomes (figure 37). During the second metaphase (figure 38), two univalent chromosomes regularly lag outside both equatorial plates. In figure 39, one of the univalents has already split. During the first and second divisions the univalents are usually distributed at random to the poles in a normal manner, but cases in which the 4 univalent chromosomes are lagging (figure 40) are frequently observed.

C. *N. Tabacum*×*alata*

The reduction division here is quite irregular. In the first division as many as 8 gemini can be distinguished (figure 41 and 43). Figure 41 shows the chromosomal arrangement at the multipolar spindle immediately after diakinesis. Figure 42 shows the next stage with the chromosomes aligned on the equatorial plate. Such figures are rather infrequent, however, for soon after this stage, the univalents pass to the poles. The first metaphase spindle most commonly seen is shown in figure 43. Eight bivalents and three univalents lie in the equatorial plate, the rest of the univalents being on the way to the poles. It seems that soon after some of the univalents have reached the poles, the bivalents begin to divide (figure 44). Figure 45 represents a situation occasionally occurring in which there has been precocious division of some of the chromosomes either during the final stages of the first division or at the beginning of the second division, the chromosomes of both plates plus the lagging one are $18+19+1=38$ instead of 32. Presumably 6 chromosomes have already divided. Such chromosomes, that is to say those which have gone through the equational division at the end of the first division, seem to behave during the

second anaphase much as do those of the Triticum hybrids (SAX 1922, KIHARA 1924, THOMSON 1926). I think that the fairly large number of lagging chromosomes found during the second division (figure 46) are not merely descendants of the few chromosomes which lagged during the first division, but also of those which have not undergone the equational division at the same time as the majority.

D. *N. Tabacum* × *Sanderae* (Cl_{10})

The reduction division in these hybrids is similar to that found in the hybrids of *N. Tabacum* × *alata*. Diads were frequently found.

E. *N. paniculata* × *Langsdorffii*

No bivalent chromosomes were observed during the various stages from early prophase to the second division. In figure 47, a heterotypic spindle, four of the chromosomes are very long and slender; but it is clear from the preparation that they are attached at both ends to spindle fibers. In a slightly later stage (figure 48), two such chromosomes are shown so securely fastened to a fiber that they seem to be pulled apart. At the end of the first division the chromosomes are regularly distributed between both poles, and during the second metaphase the number of lagging chromosomes is usually small (figure 49). An exceptional case is illustrated in figure 50 where all chromosomes lie in the same plane. The second division is virtually an ordinary equational division (figure 51).

F. *N. rustica* × *paniculata*

In the heterotypic metaphase of this hybrid the 12 bivalent chromosomes are easily distinguished from the 12 univalents. The bivalents are about twice as large and somewhat longer than the univalents, and are always found on the equatorial plate. The spherical univalents are scattered. Figure 52 shows the 12 bivalents in the center, 5 univalents on one side, 6 univalents on the other side, and one univalent overlapping one of the bivalents. The group of 5 lies in one plane, the group of 6 in another plane, while the equalorial plate with the 12 bivalents lies between them.

The arrangement of the chromosomes during the heterotypic anaphase is illustrated by figure 53. The bivalents, which divide soon after the univalents approach the poles, are found together with the univalents near the poles. Figures 54 and 55 show two sets in the second metaphase. In the first figure the chromosome number of the two plates is $18+18=36$, in the second $16+20=36$. This indicates that no univalent chromosomes

had split during the first division. This is the case for all counts made at this stage. (Cases of lagging chromosomes were also found). The second division is usually very regular (figure 56) though figures with lagging chromosomes have occasionally been observed.

G. *N. suaveolens*×*glutinosa*

During the first metaphase the chromosomes of both parents, 16+12 = 28, are scattered throughout the spindle, with no bivalents visible (figure 58). At the heterotypic anaphase the chromosomes are distributed at random to the two poles, lagging chromosomes being quite common. Figure 57 illustrates a second metaphase with 9 chromosomes in one equatorial plate and 17 in the other, while outside there are 2 lagging chromosomes. Figure 59, of the same stage, shows 4 chromosomes on a small spindle, 2 chromosomes outside, and 22 chromosomes on the equatorial plate. The chromosomal distribution during this stage is quite variable. The second division is chiefly equational. The chromosomes which lag during this division seem to be descendants of univalent chromosomes which, at the beginning of the second division, failed to reach the equatorial plates. The tetrads show a great deal of degeneration. Very often, in acetocarmine preparations, they stuck together, appeared to be shrunken, and failed to stain.

H. *N. suaveolens*×*Bigelovii*

During the heterotypic division it is very difficult to distinguish the metaphase from the anaphase, nevertheless it is easy to see that the chromosomes do not pair. The chromosomes of both parents (16+24) are scattered along the spindle. Figure 60a-b shows 37 chromosomes lying in one section and three in the other. Because the chromosome distribution in the first division is so variable the second metaphase looks quite different in different cells. Figure 61 shows 21 chromosomes lying on one plate, 16 on the other plate, and 3 lagging. Figure 62 shows the same stage in which the chromosomes are so distributed that 38 chromosomes lie on one plate, while 2 are arranged as a small spindle. The spindle fibers in this case are not visible, but the position of the chromosomes indicates a spindle. Figure 63 presents a second metaphase in which the chromosomes are distributed among three very well formed spindles, with 2 chromosomes lagging in a spindle position. The second division is regular. At this stage the chromosomes of the different spindles normally undergo the equational split simultaneously, and any lagging chromosomes which are found during and after the second division are presumably descendants

of the lagging chromosomes seen during the first division. In figure 64, showing the end of the second division, there are 8 nuclei visible. In three of the small nuclei there are 6 chromosomes; in another nucleus there are 5 chromosomes; in two other nuclei there are 10 chromosomes; while in the two very large nuclei there are 19 and 13 (?) chromosomes respectively. It is very difficult to count accurately in such a stage since the prochromosomes lie in so many different planes. I am inclined to believe that in this case, however, both of the very large nuclei really contain 19 chromosomes, the two next largest contain 10, two of the smaller ones have 6, and the other two only 5 chromosomes, originating through a chromosome distribution during the first division in which the formation of four spindles containing $19+10+6+5=40$ chromosomes took place at the beginning of the second division. The degeneration begins at the second telophase. The protoplasm is almost always very poorly fixed, the cross walls being shrunken and the nucleus itself taking the stain irregularly. It may be that the disperison of non-homogeneous chromatin between so many nuclei is due to the acceleration of the degeneration.

I. *N. rustica*×*Tabacum*, white tobacco

The first reduction division is very irregular. Although I examined many slides showing the heterotypic division there is so much variation that I could not be certain whether gemini were formed or not. In the two serial sections of figure 65 a and b, a very common situation is illustrated in which the 48 chromosomes scattered on the spindle can be easily distinguished. In some cases I have been unable to count 48 chromosomes, which I assume to be due to the presence of loose gemini.

During the second metaphase the chromosomes are distributed irregularly to both poles, some of them lagging on either side of the equatorial plates. In figure 67 is illustrated the most unequal distribution found; 36 chromosomes in one plate, 11 in the other plate, and 1 chromosome lagging between them. In anthers showing different stages of the second division, especially of the second metaphase, fairly normal single spindles were visible which were twice as large as ordinary second division spindles. Similar cases are reported by Miss LJUNGDHAL (1922) in the cross *Papaverum somniferum*×*orientale*. Such cases were thought by her to have originated through the fusion of two second metaphase spindles. Figure 66 corresponds roughly to figure 5d of her plates, though the two cases are somewhat unlike because of the different type of division. Figure 68 illustrates a case in which a cell wall has already developed between the two spindles. It is not impossible that the peculiar chromosome

distribution caused the development of the cell wall thus halting the second division just as it was starting, the result being the formation of the diads frequently found in the pollen mother cells of this hybrid. Normally the second division is very irregular, with many lagging chromosomes scattered over the two spindles. In figure 69a,b,c, the chromosomes lying in the plane of one spindle are represented in solid black, those of the other spindle in stipple, the lagging chromosomes in outline.

DISCUSSION

Inheritance in species hybrids

The gradual growth of the chromosome theory of heredity has led geneticists to interpret the anomalous distribution of characters in certain species crosses to an abnormal distribution of the chromosomes. Where the hybrids are fertile and the chromosome behavior is that which is ordinarily found in varietal crosses, the normal type of inheritance obtains (*Vide Mirabilis jalapa*×*M. longiflora*, CORRENS 1909; *Antirrhinum majus*×*A. molle*, BAUR 1911, LOTSY 1912; *Nicotiana Langsdorffii*×*N. alata*, EAST 1916). In a few cases, even partially fertile hybrids have shown a behavior similar to that found in intervarietal crosses (*Godetia Whitneyi*×*G. amœna*, RASMUSSON 1920). In general, however, one cannot go very far in a pedigree culture analysis of the results of species hybridization; though it appears probable, following STURTEVANT's work on *Drosophila melanogaster* and *D. simulans* (1920, 1921) that related species may have genes in common and may produce similar mutants.

Lack of sufficient analysis in intervarietal crosses prevents any examination of Nicotiana species hybrids after the manner used for Drosophila. SACHS-SKALINSKA (1921), however, has demonstrated Mendelian segregation for corolla color in the fertile hybrid, *N. Langsdorffii*×*Sanderae*. The colored corolla of *N. Tabacum* var. *macrophylla* which is dominant in intervarietal crosses with white tobacco has also proved dominant in the cross with *N. sylvestris*, and in somewhat diluted form in the cross with *N. Bigelovii*. The red corolla color of a *N. Sanderae* variety proved dominant over the white of *N. alata* and white tobacco. This corolla color of *N. Sanderae* was also dominant over the yellow-greenish corolla color of *N. Langsdorffii*, *N. paniculata*, and *N. rustica*. An intermediate corolla color occurred in the hybrid *N. Bigelovii*×*glutinosa* as well as in *N. Tabacum* var. *Cuba*×*alata* and *Cuba*× white tobacco. In intervarietal crosses of *Tabacum* it has been found (SETCHELL, GOODSPEED, and CLAUSEN 1922) that the petioled leaf shape is more or less dominant over

the sessile. Similarly, the petioled leaf character of *N. rustica*, *N. paniculata*, and *N. glutinosa* proved dominant in the various interspecific crosses involving these species.

The various hybrids derived by crossing species having the same chromosome number show a mixture of the parental characters due, presumably, to lack of dominance or to a similar number of dominants contributed by each parent. Where, on the other hand, the hybrids are obtained by crossing species widely differing in their chromosome number, as *N. Tabacum*×*Sanderae*, *N. Tabacum*× *sylvestris*, *N. rustica*×*Langsdorffii*, *N. suaveolens*×*longiflora*, *N. suaveolens*×*plumbaginifolia*, the hybrid resembles the parent having the larger chromosome number except in the cross *N. paniculata*×*Langsdorffii* in which the latter species appears to be dominant apart from the petioled condition of the leaves. It is not impossible that this resemblance to *N. Langsdorffii* is due to a few striking dominant characters contributed by it.

The characteristics of the sterile hybrid *N. Tabacum*×*sylvestris*, in which *N. Tabacum* is better represented, led GOODSPEED and CLAUSEN (1916) to conclude that distinct reaction systems are involved in species crosses, and that the phenomenon must be viewed in the light of a contrast between systems rather than between specific factor differences. Although they have, in some instances, recognized the influence of *N. sylvestris* on the hybrid, these authors have questioned the plate in BAUR's textbook in order to establish the complete dominance of *N. Tabacum*. KLEBS (1917), however, claims an intermediate character for the flower of this hybrid, and the intermediate corolla length of the hybrid can be distinguished in plates given by GOODSPEED and CLAUSEN (1917). Although the calcyina flower is almost completely recessive in intervarietal *Tabacum* crosses (SETSHELL, GOODSPEED, and CLAUSEN 1922), it appears in the *N. Tabacum*×*sylvestris* hybrid, albeit in not so extreme a form as in the parental types. The probability is that the twelve chromosomes of *sylvestris* do not carry the factors necessary to limit the appearance of this character, and that in general, the great resemblance to *Tabacum* is due to the transmission to the hybrid of a larger number of dominant factors with the larger number of chromosomes. The high degree of sterility, on the other hand, depends (EAST 1915, 1921) on the interrelation between the chromosomes.

A species hybrid parallel to that cited by Goodspeed and Clausen is found in crosses between white tobacco and two different *N. Sanderae* plants. The hybrids obtained show a high degree of resemblance to the *N. Tabacum* parent, but corolla color of the *Sanderae* parent is dominant.

Non-viability of the gametes in species hybrids

In the matter of sterility through the production of non-viable gametes, it is necessary to distinguish between completely sterile and partially sterile hybrids. The first category is the one most frequently found in *Nicotiana*; the second I have found only in *N. rustica*×*paniculata*. From cytological studies of different hybrids of the first category, such as *N. Bigelovii*×*suaveolens*, *N. suaveolens*×*glutinosa*, and *N. paniculata*×*Langsdorffii*, complete sterility seems to be the logical expection, since no pairing of chromosomes occurs during the first division. The chromosomes are distributed irregularly between the two poles of the heterotypic spindle, some being left behind; or, as in *N. Bigelovii*×*suaveolens*, they are distributed among three or four homeotypic spindles. In *N. rustica*×*paniculata*, on the other hand, there is distinct pairing of the chromosomes. Presumably this should afford a greater expectation of the production of viable gametes, though there are cases known where a seemingly normal reduction does not lead to viable gametes.

Of some interest is the relation between the chromosome behavior and the morphological appearance of the hybrids. *N. paniculata* and *N. Langsdorffii* both belong to the *rustica* section, but they produced very weak hybrids with no pairing of chromosomes. It seems that the mode of the reduction division is not correlated with the vigor of the hybrid, nor even with the relationship between the species, although this case is somewhat questionable as the accepted taxonomic position of *Langsdorffii* is open to criticism (Lock 1909). *N. suaveolens* and *Bigelovii*, both of the *Petunioides* section, must have some degree of genetic compatibility as demonstrated by the extreme vigor of their hybrids, but again there was no pairing of chromosomes in the reduction division, In the hybrid *N. suaveolens*×*glutinosa* I again found hybrid vigor and non-pairing of chromosomes, but here the parents are not closely related taxonomically.

It is to be noted that regularly in species hybrids of Triticum and other genera, the longitudinal split of the univalent chromosomes occurs during the first division (KIHARA 1924, SAX 1922, THOMSON 1926); but in Nicotiana in all the species hybrids studied no longitudinal split of the univalent chromosomes occurred during the first metaphase.

Non-viability of the zygote in species hybrids

Sterility in plant and animal hybrids is sometimes due to the fact that the zygote dies during its development. Such cases are found very frequently in Drosophila, where they are due to definite lethal or semi-

lethal factors (BRIDGES, MORGAN and STURTEVANT 1925). I have observed some interesting cases of zygote mortality in certain Nicotiana hybrids, such as *N. suaveolens*×*Tabacum* var. *macrophylla*. The cross is easily made and the germination of the seeds is nearly normal. After germination the seedlings start their growth at a rapid rate with a tendency to hybrid vigor until the first true leaves appear. At this stage the seedling growth stops, and after a time the plants die. This phenomenon was confirmed by three or four successive sowings. The leaves of the seedlings were a normal green as if no disturbance had occurred in the chlorophyll development. The root system, however, which was quite normal at the beginning, began to degenerate rather early although the leaves remained green a long time after. This was characteristic of all hybrids which died during vegetative development. This same cross was successful for SAGERET and for GÄRTNER, and their plants reached maturity. It is rather peculiar that in the reciprocal cross the pollinated *Tabacum* flowers dropped soon after pollination, although crosses were almost always successful when using species with the higher chromosome number as female.

The hybrid *N. Tabacum* var. *macrophylla*×*glutinosa* stopped its growth in a later stage, after from 5 to 10 leaves had developed. The root system which was very well developed degenerated with the cessation of growth, but long after that the plant was still green. I was inclined to believe that some pathological organism was responsible for the death of the roots, although the neighboring plants of other crosses were normal. I cut away the old root of several plants, disinfected them in Uspulun, and grew them in sterilized soil under artificial light 3–4 hours during the night, as the day was very short at that time. The plants recovered, and after only one week I was able to see a new root developing normally. New leaves were formed, and in three weeks the plants came into flower. Sister plants growing without artificial light never came to flower. It seems that for some reason metabolism was abnormal, and that this defect was partially nullified by increased exposure to light. This success encouraged me to try defective plants of hybrid combinations under similar conditions, and I was especially hopeful of the hybrid *N. Tabacum* var. *macrophylla*×*plumbaginifola*; but had no success. Thirty plants from two sowings were observed. In both cases the seedlings started with a very rapid growth, but stopped after the 3d or 4th leaf developed.

The single seedlings obtained from *N. Tabacum* var. *macrophylla*×*Langsdorffii* and from *N. Tabacum* var. *macrophylla*×*longiflora* had deformed cotyledons and no visible growing point. The same *N. paniculata*

plant was pollinated by three different *alata* plants and in two of the crosses the seedlings died after developing the fourth true leaf. In this case I am not sure whether they died because of their genetic constitution or because of the greenhouse conditions during the winter, or, perhaps, a combination of both. One plant of *N. Tabacum* var *Cuba* was pollinated by two different *alata* plants and by a *Sanderae* plant. With one of them, *alata*, No. 20, three capsules were obtained, but not a single seed germinated. With *alata*, No. 25, the seedlings produced died in different stages during their development and only two finally reached maturity. With *Sanderae*, No. 16, four seedlings reached maturity. The chromosomes were counted in the root-tips of some of the hybrids seedlings of *cuba*×*alata* No. 25, which died after developing the fourth true leaf and the number was found to be over 30.

From the cross *N. Tabacum* var. *macrophylla*×*nudicaulis* I obtained plump seeds having endosperms, but which did not germinate. I do not know whether the seeds contained embryos or not, as I did not make a histological investigation.

Incompatibility between species in Nicotiana

The degree of compatibility between different species is supposed to be indicated by their taxonomic relationship. This study has shown that, with some exceptions, hybridization is successful more frequently when the species with the higher chromosome number is used as female. While these results are not entirely in accord with those of other investigators, I believe that this correlation holds in Nicotiana. The literature of genetics affords no clearly parallel case. From the hybridization results between the various Triticum species as reported by different investigators, it is not clear in which direction the cross is easier, but it is evident that the species with the lower chromosome number is generally used as the female parent. Of the 26 crosses reported (TSCHERMAK 1914, SAX 1922, KIHARA 1924) in only 4 is the species with the higher chromosome number used as the female. KIHARA reports successful pollination of a species in the Emmer group (n = 14) by one of the *monococcum* group (n = 7). The other three cases are reported by SAX who successfully pollinated three species of the *vulgare* group (n = 21) by three of the Emmer group. Similar results have been reported for Papaverum species crosses, where 4 out of 17 hybrids (FOCKE 1881, LJUNGDAHL 1924) were produced by using pollen of species with the higher chromosome number. Unfortunately, these investigations do not make clear just what crosses were attempted, and which were successful.

CONCLUSION

The chromosome numbers of the different Triticum species (SAKAMURA 1918, SAX 1918) have been found to be what should be expected from the taxonomic arrangement of the species, the interspecific hybridization results, the serological studies, and resistance to parasites. The three sections of the genus are represented by three different numbers, each found only among the species of a given section, and the three numbers establish the polyploid series 2x, 4x, 6x, with the basic number 7. In Fragaria (LONGLEY 1926, ICHIJIMA 1927) the situation is similar. In Nicotiana, however, as in Chrysanthemum (TAHARA 1921), and Rosa (TÄCKHOLM 1922), each taxonomic section contains more than one characteristic chromosome number. Species with an aberrant number of chromosomes in a polyploid series, as in *longiflora* and *plumbaginiolia* in Nicotiana are frequently observed in various genera. KUWADA (1919) assumed for maize that transverse segmentation of some of the chromosomes is a genetically fixed phenomenon. HEILBORN (1924), however, believes that in Carex the higher chromosome number in various related species arose practically always through non-disjunction of single whole chromosomes rather than through fragmentation; and that hybridization was an important factor in the perpetuation of the new chromosome number. If transverse division is to be taken as the explanation of the origin of some Nicotiana chromosome numbers, one may explain the 10 haploid chromosomes of certain species as arising by the occasional transverse division of two long U-shaped chromosomes, and the 12 chromosome type by the transverse division of four long U-shaped chromosomes of certain 8 chromosome species. It is to be noted, however, that the 12 chromosome species have a large number of U-shaped and very long chromosomes.

WINGE's (1917) theoretical interpretation of the origin of new forms by the reduplication of all the chromosomes of two species after hybridization is probably important in connection with Nicotiana species (CLAUSEN and GOODSPEED 1925). The morphological appearance of certain related Nicotiana species indicates that they have a common origin. *Rustica* and *paniculata* differ noticeably only in respect to corolla length and shape, for example. *Nudicaulis* and *trigonophylla* are also similar to each other, and also show the same chromosome relation, 24:12. The three species *glutinosa*, *tomentosa*, and *Rusbyi* show a gradation in the morphological characteristics of the two sections *Tabacum* and *Rustica*, *glutinosa* showing largely *Rustica* characters, *Rusbyi* largely *Tabacum* characters, with *tomentosa* standing in between. Such instances point to hybridization as a possible origination of species.

The possibility that the species have originated as multiples from a type having the basic number 4, might be indicated if the reduction division of any of the hybrids showed this number of bivalents among the univalents or *vice versa*, as has been found in Triticum (SAX, K., and SAX, H., 1924); but no such cytological situation has been found.

SUMMARY

1. Nineteen species of Nicotiana have been studied cytologically, and species with the following haploid chromosome numbers were found: 8, 10, 12, 16, 24. All of these numbers have occurred among the species belonging to the Petunioides section. Among the species of the *Rustica* section, the haploid numbers found were 8, 12, and 24. Differences in shape and size were found among the chromosomes of the different species and in certain cases among the chromosmes of a single species. Counts made in the root-tips confirmed the numbers found in the pollen-mother cells. There are great differences in size and shape between the chromosomes of the somatic cells and those of the pollen-mother cells, the first being very long, entwined, and generally U-shaped, while the latter spherical or bean-shaped.

2. Fifteen species were crossed with the view of discovering if any parallelism exists between their taxonomic relationship and their ability to hybridize. Four degrees of compatibility between the species were distinguished according to the results obtained in the hybridization experiments: (1) Crosses in which the flowers pollinated dropped after the pollination; (2) those in which capsules were produced without seeds, or with seeds which did not germinate; (3) those in which the seedlings died before reaching maturity, and finally (4) those which produced mature hybrids. It was found further that the species with 8 haploid chromosome numbers produced fertile hybrids (as did species with 10 haploid chromosomes); but the ability to hybridize was very low between the species with 12 chromosomes, though less difficult between the species with 24 chromosomes. In crosses between species differing in their chromosome number, success was rare when species with a lower chromosome number were used as female; and in the cases where the cross succeeded in both directions, the percentage of successes was less when the female parent had the lower chromosome number.

3. A morphological description of the species hybrids obtained is presented. In crosses between species with the same chromosome number, the fertile hybrids, generally speaking, were intermediate, but

the great variability of the F_2 plants and the obvious Mendelization of the striking qualitative differences leads one to believe that the genes are distributed as in ordinary varietal crosses. In hybrids between species differing in their chromosome number, the parental type with the greater number of chromosomes was better represented morphologically.

4. In nine of the species hybrids, the reduction division in the pollen-mother cells was studied. Four types of division were found. In *N. alata*×*Langsdorffii* (8×8), the reduction was normal, no univalents being found. In *N. longiflora*×*Sanderae* (10×8), *N. Tabacum Cuba*×*alata* (24×8), *N. Tabacum* white×*Sanderae* Cl_{10} (24×8), and *N. rustica*×*paniculata* (24×12), the number of bivalent chromosomes was equal to the haploid chromosome number of the parent with the lower chromosome number. In *N. rustica*×*N. Tabacum* white tobacco (24×24) a few loose pairs appeared to be present. In *N. paniculata*×*Langsdorffii* (12×8), *N. suaveolens*×*glutinosa* (16×12), and *N. suaveolens*×*Bigelovii* (16×24), no pairing of chromosomes occurred.

In cases in which a regular pairing of chromosomes was observed, the univalents were usually distributed to both poles without splitting during the heterotypic division, though occasionally lagging chromosomes were found. In cases where no pairing was observed, the chromosomes were distributed irregularly to the poles; and in some few cases, particularly in hybrids between species with high chromosome numbers the occurrence of three spindles during the second metaphase was observed. The reduction division of those hybrids in which some few loose pairs occurred during the heterotypic metaphase or before, was most complicated and irregular; and this type of irregularity was observed in a hybrid where the total number of chromosomes was high (24×24); here diads were observed among the tetrads.

The second division was always a regular equational division.

5. The bearing of these results on certain theoretical problems is discussed. Where the reduction division is normal, inheritance appears to be normal. Where the number of chromosomes contributed by the two parents is identical and the reduction division is abnormal, the hybrids show an intermixture of the characteristics of the two parents, presumably because of lack of dominance or because an approximately equal number of dominants is contributed by each parent. Where the number of chromosomes contributed by the two parents is unequal, the hybrids tended to resemble the parent with the higher chromosome number, with but few exceptions. In these exceptional cases, perhaps the con-

tribution of a few striking characters by the species having the lower chromosome number tends to distort the result.

6. The hybrids which reached maturity were completely sterile, with the exception of *N. rustica*×*paniculata*; and this hybrid was partially sterile. Non-viability of the gametes seems to parallel irregular reduction division.

7. The compatibility of the species shows a greater relation to the chromosome number than to the taxonomic status, but the mere fact that two species have the same chromosome numbers does not mean that they will cross. In general a cross can be made more easily when the species with the higher chromosome number is used as the female parent.

8. There is no particular correlation between the vigor of the hybrid and the mode of the reduction, except that in cases where the reduction division is normal, the plants are never weak. Cases were found in which the hybrid was weak although chromosome pairing occurred, as in *N. Tabacum*×*alata*. On the other hand, in *N. Bigelovii*×*suaveolens*, where no chromosome pairing occurred, the hybrid was very vigorous.

9. Weak hybrids can sometimes be aided in their development by a change in external conditions, such as an increase in the relative amount of light.

10. Though the Nicotiana species probably did not originate by multiplication of 4 chromosomes, it is possible that they did originate from one or more 8-chromosome species by a reduplication of certain chromosomes or of whole sets of chromosomes.

After this paper was completed, there was an opportunity for studying cytologically the following two species of the genus:-

N. caudigera Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised are large, branched, with long petioled leaves, cordate below and lanceolate above, having spine-like glandular hairs on the surface and on the margin. The corolla is white with broad cylindrical tube and short, shallow rounded lobes on the limb. In the pollen-mother cells, stained with acetocarmine, the dumbbell shaped chromosomes are constricted so that their number in some stages appears higher than the real one—$x=12$. This was clear on permanent slides where, presumably due to the fixation, the chromosomes were shortened, less closely arranged, and their constriction invisible in the deep hematoxylin stain. The root tip cells contain 24 slender, straight or U-shaped chromosomes

N. solanifolia Wolf–*N. cardiophylla* Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised developed slowly to a short non-branched stem, with many long petioled, broadly ovate and highly undulate leaves. The corolla resembled that of *N. paniculata*, except for being larger and of cream yellow color. The pollen-mother cells stained with acetocarmine, show 12 chromosomes and the root tip sections on the permanent slides show 24 straight or U-shaped chromosomes. Under the name *N. solanifolia* were raised plants from seeds obtained from a commerical source which were quite different from those described above, and resembled more closely *N. rustica* var. *humilis*, except that the corolla was even smaller, somewhat different in shape and slightly violet-tinged on the tube. Their chromosome number was $x = 24$ or $2x = 48$.

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Description of Plates

The drawings except figures 60, 65, 69, were made from single sections with the aid of a camera lucida. All figures in plates I and II were drawn by using the large Zeiss microscope with ocular 10 and oil immersion 1/16. Those of plates III and IV by using compensating ocular 12 instead of 10. As reproduced, all figures have a magnification of 1500 diameters.

Plate 1

Figures 1–15 represent the polar view of the mitotic metaphase of the following *Nicotiana* species:

$2x=48$

Figure 1.—*N. Tabacum*, var. *macrophylla*.
Figure 2.—*N. rustica*, var. *brasilia*.
Figure 3.—*N. Bigelovii*.
Figure 4.—*N. nudicaulis*.

$2x=32$

Figure 5.—*N. suaveolens*.

$2x=24$

Figure 6.—*N. glutinosa*.
Figure 7.—*N. sylvestris*.
Figure 8.—*N. paniculata*.
Figure 9.—*N. glauca*.
Figure 10.—*N. tomentosa*.
Figure 11.—*N. trigonophylla*.
Figure 12.—*N. Palmeri* (?)

$2x=20$

Figure 13.—*N. plumbaginifolia*.

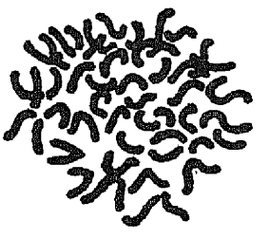
$2x=16$

Figure 14.—*N. alata*.
Figure 15.—*N. Langsdorffii*.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 1



1



2



3



4



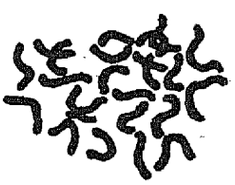
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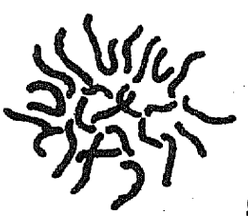
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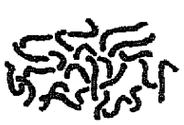
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10



11



12



13



14



15

PLATE 2

Figures 16–33 represent the polar view of the heterotypic metaphase of the following Nicotiana species: $x=24$ Chromosomes

FIGURE 76.—*N. Bigelovii.*
FIGURE 17.—*N. quadrivalvis.*
FIGURE 18.—*N. rustica*, var. *humilis.*
FIGURE 19—*N. Tabacum*, var. *macrophylla.*
FIGURE 20.—*N. viscosa* (?)
FIGURE 21.—*N. nudicaulis.*
$x=16$ chromosomes
FIGURE 22.—*N. suaveolens.*
$x=12$ chromosomes
FIGURE 23.—*N. glauca.*
FIGURE 24.—*N. sylvestris.*
FIGURE 25.—*N. paniculata.*
FIGURE 26.—*N. glutinosa.*
FIGURE 27.—*N. acuminata.*
FIGURE 28.—*N. trigonophylla.*
FIGURE 29.—*N. Palmeri* (?)
$x=10$ chromosomes.
FIGURE 30.—*N. longiflora.*
$x=8$ chromosomes.
FIGURE 31.—*N. Langsdorffii.*
FIGURE 32.—*N. alata.*
FIGURE 33.—*N. alata.*
FIGURE 34.—*N. alata*—homeotypic metaphase.
FIGURE 35.—*N. alata*—homeotypic metaphase.
FIGURE 36.—*N. alata*—heterotypic metaphase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 2

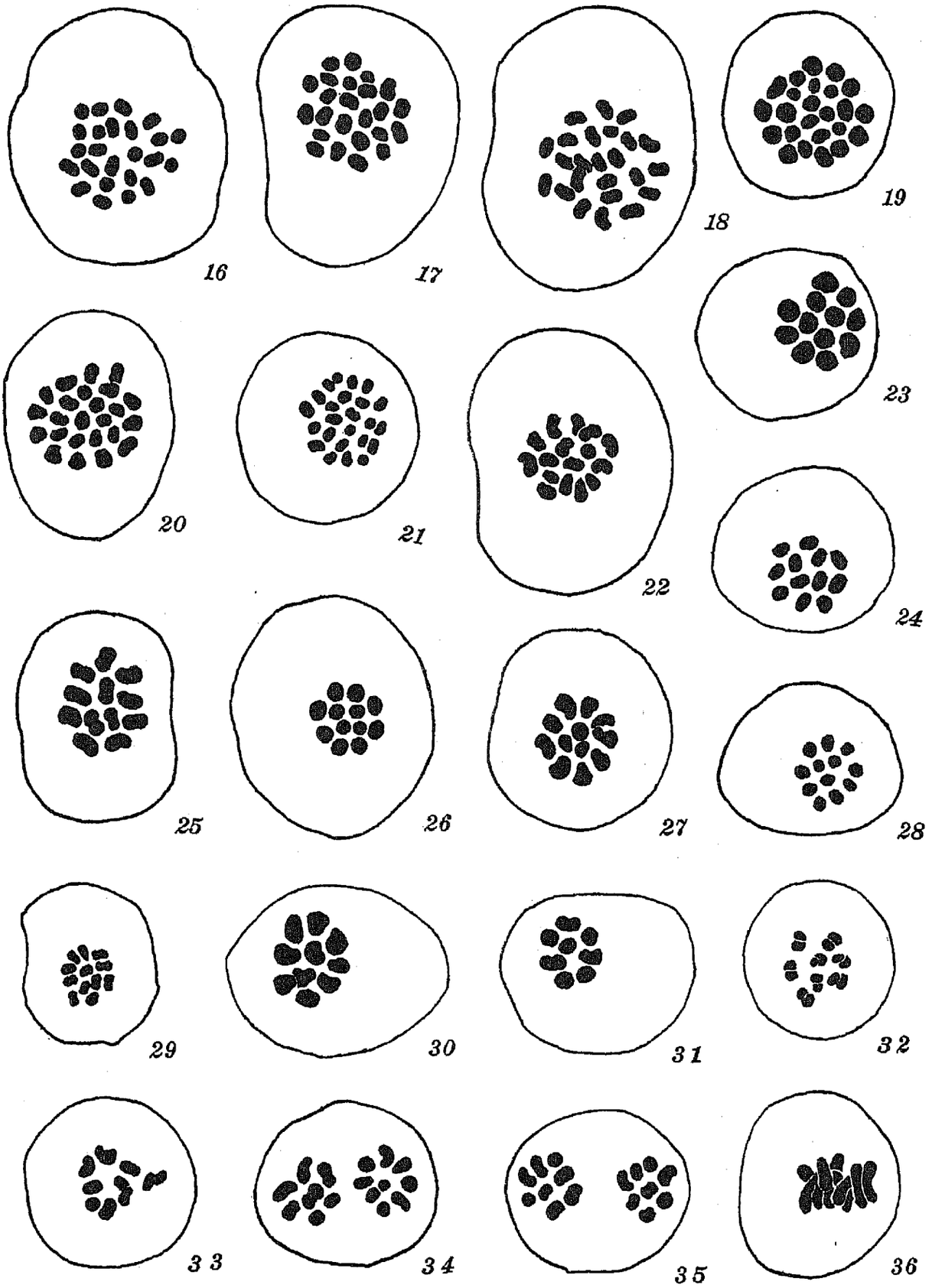


PLATE 3

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. longiflora ($x=10$)×*Sanderae* ($x=8$)

FIGURE 37.—heterotypic metaphase, side view.
FIGURE 38.—homeotypic metaphase.
FIGURE 39.—homeotypic metaphase.
FIGURE 40.—homeotypic anaphase.

N. Tabacum ($x=24$)×*alata* ($x=8$)

FIGURE 41.—very early heterotypic metaphase, polar view.
FIGURE 42.—very early heterotypic metaphase, side view.
FIGURE 43.—heterotypic metaphase, side view.
FIGURE 44.—late heterotypic metaphase.
FIGURE 45.—homeotypic metaphase, polar view.
FIGURE 46.—homeotypic anaphase, side view.

N. paniculata ($x=12$)×*Langsdorffii* ($x=8$)

FIGURE 47.—heterotypic spindle.
FIGURE 48.—heterotypic anaphase.
FIGURE 49.—homeotypic metaphase, polar view.
FIGURE 50.—homeotypic metaphase, polar view.
FIGURE 51.—homeotypic anaphase, side view.

N. rustica ($x=24$)×*paniculata* ($x=12$)

FIGURE 52.—heterotypic metaphase, polar view.
FIGURE 53.—heterotypic anaphase, side view.
FIGURE 54.—homeotypic metaphase, polar view.
FIGURE 55.—homeotypic metaphase, polar view.
FIGURE 56.—telophase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 3

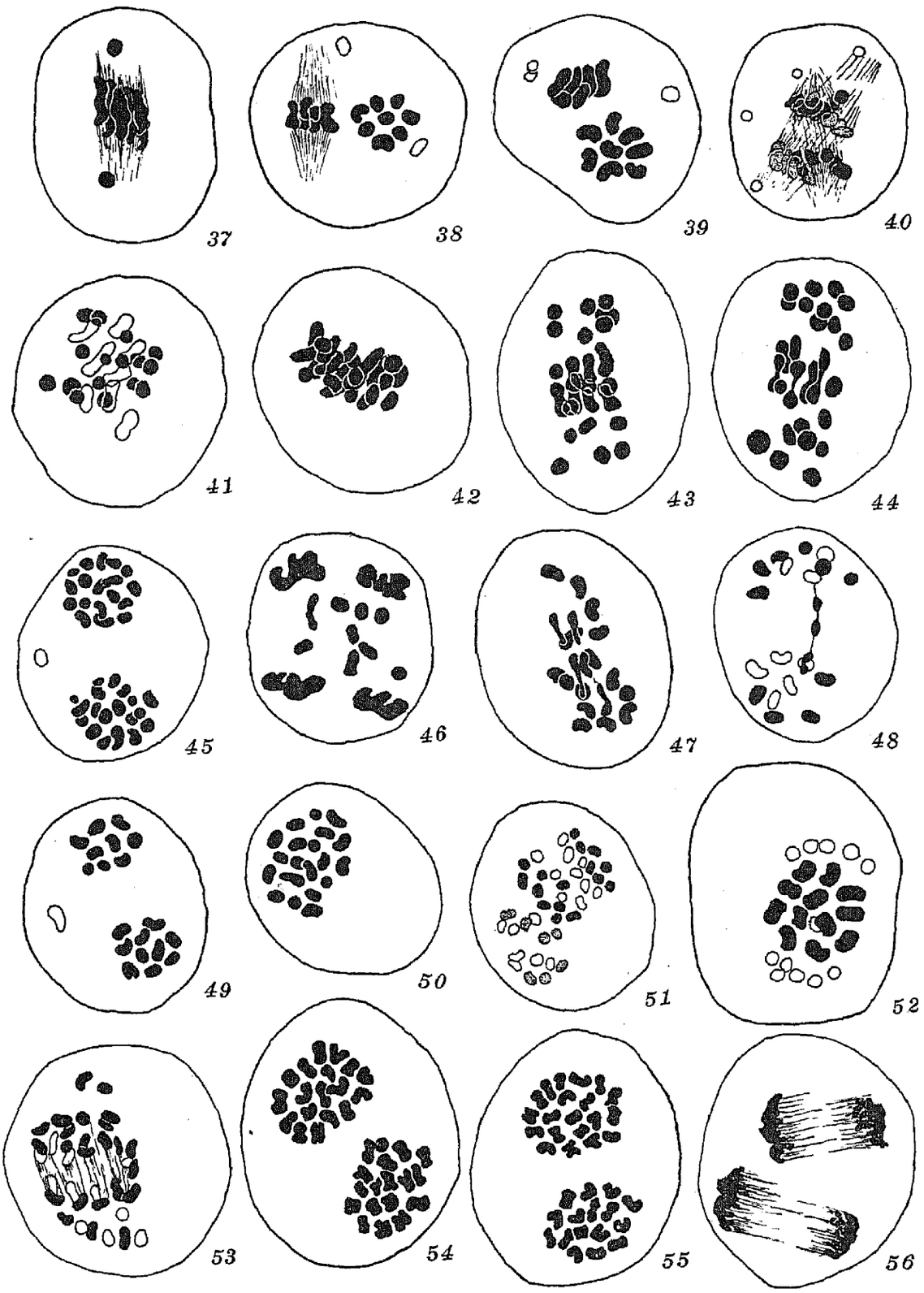
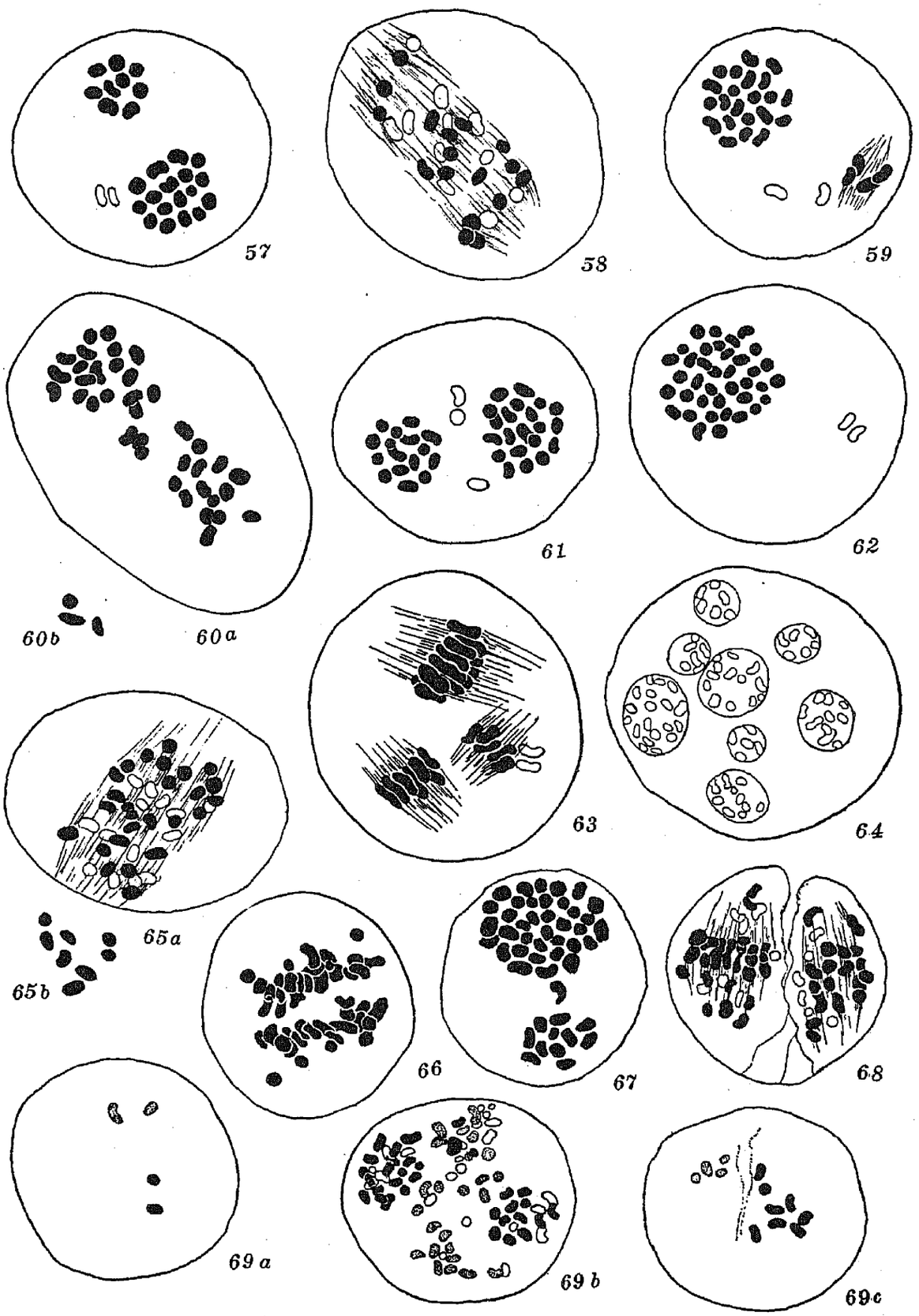


PLATE 4

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. suaveolens ($x=16$)×*glutinosa* ($x=12$)
FIGURE 57.—homeotypic metaphase, polar view.
FIGURE 58.—heterotypic metaphase,side view.
FIGURE 59.—homeotypic metaphase.
N. suaveolens ($x=16$)×*Bigelovii* ($x=24$)
FIGURES 60a & b.—heterotypic spindle.
FIGURE 61.—homeotypic metaphase, polar view.
FIGURE 62.—homeotypic metaphase, polar view.
FIGURE 63.—homeotypic spindles.
FIGURE 64.—octad.
N. rustica ($x=24$)×*Tabacum* var. *alba* ($x=24$)
FIGURE 65a & b.—heterotypic spindle.
FIGURE 66.—homeotypic anaphase.
FIGURE 67.—homeotypic metaphase, polar view.
FIGURE 68.—homeotypic metaphase, side view.
FIGURE 69, a, b, c.—homeotypic anaphase, polar view.

CHRISTOFF M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 4



THE MEASUREMENT OF GENE MUTATION RATE IN DROSOPHILA, ITS HIGH VARIABILITY, AND ITS DEPENDENCE UPON TEMPERATURE[1]

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[1] Department of Zoology, UNIVERSITY OF TEXAS, Contribution No. 211.

THE PROBLEM

THE FAILURE OF ATTEMPTS TO "PRODUCE" GENE VARIATION[2]

Biologists are in general agreed that the basic problems of organic evolution are concerned largely with the nature, the causation, and the modes of transmission of heritable variations. Great have been the strides of the last quarter century in our understanding of the last mentioned phenomena—those relating to the *transmission* of variations—owing chiefly to the growth and ramifications of the gene and chromosome theories, and these advances, together with the manifold discovery of "mutations" of these genes, have profoundly affected our viewpoint on the former questions. In accordance with these findings, most geneticists at present conceive of heritable variations (at least, of most of those heritable variations which might be of importance in evolution) as consisting in sudden, rare, discrete changes in individual genes, and the problem of variations thus becomes transferred from the germ plasm as a whole, or even from the organism as a whole, to a much minuter (ultramicroscopic) portion of it. Nevertheless, this being admitted, there is nearly as great a lack as ever of positive knowledge concerning the questions first mentioned—namely, the real nature of heritable variation in that material in which it does occur, and the factors causing, conditioning, or influencing that variation.

Numerous claims, have, to be sure, been made, ranging from assertions concerning the general heritability of the effects of training to such specific theses as that of the induction of a given mutation by means of an antibody. And more such claims are being made, almost daily. It is to be noted, however, that the respectability of such claims is in almost direct proportion to their newness; none of those which has had the opportunity to withstand the test of many years, of critical analysis, and of repeated trial, has succeeded in doing so. For the pilotage of modern genetics is essential to steer clear of the mines of heterozygosis and recombination, of delayed or "maternal" inheritance, of varying differential viability, and of unconscious or even "accidental" selection. Exceptional care is sometimes necesssary also to avoid a systematic repetition, in particular lines of descent, of certain environic modifications which may

[2] The present paper was written in the fall of 1926, just prior to the author's mutation experiments involving X-rays (a preliminary account of which has been given in SCIENCE, July 22, 1927). The discussion in the present paper, therefore, must nowhere be taken as applying to this later work, the results of which deviate widely from those of previous mutation experiments. Nevertheless, it is believed that the points made in the present paper still hold, within the limits thus set.

be caused by contagious disease, by special treatment, or by other conditions. The fluctuating personal equation and means of detection employed in the finding of the variations is another important factor. Further, to analyze the results genetically, when obtained, requires special methods. In addition, an elementary knowledge of the theory of probability is usually prerequisite in order to avoid being misled by the mirage of non-significant numerical differences. Sometimes not all of these dangers have been overlooked, but after a careful survey of all this literature on allegedly induced variation, the present writer has been led to the opinion that in none of the reported cases, not even in the recent ones, have any changes of the genes been demonstrated to have been brought about by treatment,[3] nor does such an effect seem to me to have been made even reasonably probable, in the light of genetic analysis. On the other hand, in a fair number of recent cases (e.g. MANN 1923) adequate technique for avoiding the above common sources of error has been employed, and in these very cases the results of treatment, so far as could be determined, were all admittedly negative. It is in this sense that "positive" knowledge may be said to be lacking.

For all that, gene change most certainly does occur sporadically, having been demonstrated most frequently in the more numerous "untreated" cultures used in breeding experiments primarily concerned with gene distribution,—not to mention the cases of mutated genes that have been found in nature. Do the preceding results mean, then, that mutation is unique among biological processes in being itself outside the reach of modification or control,—that it occupies a position similar to that till recently characteristic of atomic transmutation in physical science, in being purely spontaneous, "from within," and not subject to influences commonly dealt with? Must it be beyond the range of our scientific tools?

THE NEED FOR A MEANS OF MEASURING MUTATION RATE

We need be forced to no such hopeless conclusion as might above seem indicated. The simple reason is that the "rate" of mutation, that is, of readily detectible mutation, is probably so low under ordinary circumstances in most of the organisms dealt with in the experiments hitherto carried out, that a 100 percent, or even a 500 percent effect upon it, due to a given treatment, would, with the methods and number of individuals that have been employed, be very likely to escape detection. For ex-

[3] We may except here the case of variegated corn, in which only a single, especially mutable, genetic locus is concerned. We are also excepting the preliminary experiments of ALTENBURG and the present writer, on temperature, which led up to the present work.

ample, in certain experiments in which mutations have been looked for in *Drosophila melanogaster* (popularly supposed to be so exceptionally mutable), scarcely one mutant has been found among 50,000 flies; a figure of the same order of magnitude would be reached by dividing the several hundred (400±) mutations found in the collective work on these flies into the twenty million (more or less) flies of this species that have, all in all, been examined. An experiment comprising 10,000 flies is usually considered respectable, yet it will be seen that such a count might easily fail to reveal a single mutation, even though, owing to a given treatment with, say, radium or alcohol, the usual tendency to mutation had been exceeded a thousand percent! On the other hand, if 2 or 3 mutations had been obtained here, this small figure would be practically meaningless, even if personal equation and the other sources of error could be allowed for. In mammals, much smaller numbers are usually dealt with; here, then, a hundred fold increase of the mutation rate, due to treatment, might well escape discovery (supposing that in them mutations ordinarily occurred and could be detected with the same frequency, per animal counted, as in flies).

Previous experiments, then, not only seem inadequate to prove that environic agents "produce mutations," but they also fail to prove that such agents do not "produce mutations,"—if in the word "production" we may include the idea of the causation of a radical increase of frequency of the process (inasmuch as this would involve the appearance of many mutants that otherwise would have been non-existent). All that the experiments do indicate is that mutations cannot, by means of most of the agents and with the dosages used, be produced *en masse*, in such vast numbers as to exceed the ordinary mutation rate perhaps a hundred fold (10,000 percent).

It may be emphasized again here that no ultimate distinction exists between the idea of "production" as such and the idea of a change in rate of occurrence. If, however, mutation is a physical or a chemical reaction depending on certain disturbances of molecular stability that can occur to an appreciable extent even under "ordinary" conditions, it may be very useful to consider it in the manner above suggested, that is, in terms of its rate, just as we consider many chemical reactions in this way, even though mutation must have a vastly slower rate than most reactions with which the chemist deals. The question as to "what agents will produce mutations" may then be changed to read, "what agents will cause a noteworthy change in mutation rate?"

We must certainly consider as "noteworthy" in the present connection

such a change as a doubling of the rate (100 percent increase), just as this would be considered significant in the case of any other kind of reaction. A change of this magnitude in the rate of mutation might be of significance both in its bearing on the nature of mutation and of the mutating gene material and in relation to the process of evolution. The finding of such a change, morever, might be but an entering wedge. For if, by systematic and precise investigations, agents were discovered that produced increases of the above order of magnitude in the mutation rate, it might be that eventually, by combining them, or applying them in special ways, methods might be arrived at whereby vastly greater effects than those first obtained could be achieved, and so the ideal of the "production of mutations" (*en masse*) might finally be realized.

It will now be evident, however, that a new technique will be desirable for attacking the problem of the variation of the hereditary units from this angle. A method is called for whereby enough mutations can be discovered to yield a figure for mutation rate in a *control* series of individuals, which will be large enough, in absolute numbers, so that, when it is compared with a corresponding figure obtained from the treated series, a difference of about 100 per cent, at least, could be recognized as being a "real" or "significant" difference rather than a mere "chance difference" or "error of sampling." This figure of 100 per cent is tentatively chosen because in some already known processes within the organism, for instance some reactions of "basal metabolism," larger changes in rate than this are not commonly produced, while at the same time changes as large as this should be attainable for most chemical reactions.

The idea of developing such a technique, for bisexual organisms at any rate,[4] does not seem to have suggested itself before the experiments preliminary to the present series were undertaken, for the seeming extraordinary rarity of mutations, even in Drosophila, apparently put them beyond the pale of such quantitative measurement. The task of actually counting mutations in ordinary cultures, in order to compare their frequencies of occurrence there with those under other, contrasting conditions would have seemed almost like that of counting needles in haystacks, to compare their frequencies, or like making graphs to show the rates of occurrence of gold pieces on streets of different types. The

[4] In the case of BAUR'S recent experiments with self-fertilized lines of *Antirrhinum* the frequency of detectable mutations appears considerably higher than in other organisms examined; this is doubtless due in part to the fact that selfing makes possible, after just one generation, the manifestation of all recessive genes for which an individual may be heterozygous. On the other hand, the relatively high "mutation" rates reported for *Oenothera* are, as has been abundantly shown, due to gene and chromosome recombinations rather than to real changes in the genes.

objective of most genetic counts, therefore, was the determination of the frequencies of crossing over, of chromosome reassortment, of non-disjunction, and of other phenomena connected with the transmission rather than with the origination of gene variations. Meanwhile, mutations were of course recorded as they arose, but the numbers in which they were found were insignificant in any one given experiment, and still meant little even when many different experiments were totalled, because of the fact that the conditions for their detection varied so greatly from one experiment to another—owing to personal equation, to the differing characters being considered, to the different methods of breeding used, the varying external conditions, diverse stocks, etc.

LETHALS AS A PRACTICABLE INDEX OF MUTATION RATE

It had long been held, however, by the present writer, on the basis of theoretical considerations (MULLER 1917, 1918), that the number of mutations resulting in lethal genes probably greatly exceeds the number resulting in genes that cause visible, readily detectable character changes. If this were true, then, counts of the number of *lethals* arising by mutation might yield figures high enough to make mutation rate capable of quantitative study in the sense previously explained. Differences in mutation rate thus discovered could in all probability be generalized, to apply to visible mutations as well, since there are both theoretical and experimental reasons for believing that most lethals, as a class, differ from other genes only in the more drastic and disastrous end results they happen to produce in embryogeny, and not in their essential nature, or in their mode of origin by mutation.

The correctness of the idea of a relatively high lethal mutation rate was then proved by ALTENBURG and by the present writer, both in separate and in joint work (only a fraction of which has yet been published, and that only in the form of preliminary notes). This work will be referred to in somewhat more detail later; it will suffice here to say that the number of lethals that arise was found to be far in excess of the number of "visible mutants," both in the X- and in the second chromosome of *D. melanogaster*; the frequency of origin of the lethals was so high, in fact, that it immediately seemed evident that a quantitative study of mutation, using the frequency of origin of lethals as the index of mutation rate, was indeed possible. Another advantage of using lethals lay in the fact that "personal equation" was thereby largely eliminated: observers will often disagree or be uncertain regarding the existence of a "visible" mutation, whereas, with lethals, detection is objective and there are relatively far fewer border-line cases (of "semi-lethals").

Even with these advantages, however, the work with lethals was laborious, and further serious difficulties and apparent inconsistencies arose, which will be described. It has been the chief aim of the present writer's work during the past 8 years to develop a technique that will overcome these difficulties, and by the use of it, to obtain decisive counts that would establish the effectiveness, or the non-effectiveness within certain limits, of some important environic influence in modifying the rate of gene mutation. It is believed that this object has at last been definitely achieved, and that the data so obtained in the first place furnish information of theoretical (perhaps ultimately of practical) value, and, in the second place, demonstrate the general usefulness of the method for an unlimited amount of further work on the rate of gene mutation under varying external and internal conditions.

TEMPERATURE AS THE CONDITION FIRST TO BE INVESTIGATED

The environmental condition which was first chosen for the study of its effectiveness in altering the rate of gene mutation was temperature. This was decided upon for a number of reasons. There was, of course, the important practical reason that temperature differences are relatively easily produced and maintained. Another reason was that heat is bound to penetrate the organism of *Drosophila* and there to alter decidedly the rate of nearly every chemical reaction: for example, a rise of 10°C, to which it is quite practicable to subject the flies, causes chemical reactions to rise to between 2 and 3 times their former speed. Hence, if mutation involves a chemical reaction it might well be expected to increase in frequency at least 100 percent, with a rise of 10°C, unless its rate is somehow restricted by that of some physical process, or unless there is involved in addition some chemical process or processes of an antagonistic nature, that are accelerated more than those processes which induce mutation. But if mutation is controlled by some "*entelleche*," or "vital force," or "perfecting principle" (see "rectigradation"), or if its rate depends wholly on some unguessed magnetic, sub-atomic, or other physical force little affected by the kinetic energy of molecules, it could scarcely be expected to respond so strongly to temperature change.

Data on effects of temperature would have another point of contact with other problems inasmuch as the rate of evolution must necessarily be limited, for one thing, by the rate of mutation; in fact, given rigorous selection, with other factors equal, these rates would be proportional to one another. Since great differences in temperature are common in nature the experimental results should therefore inform us concerning the ef-

fectiveness of one possibly important natural factor in the differing rates of evolution of different groups of organisms. Then, too, if the answer was positive (that is, heat found effective), temperature regulation might be used to advantage in some practical breeding work, either for the purpose of stabilization, or of promoting variation. Finally, a positive result with temperature could rather readily be followed up in various ways in subsequent experiments, in further analysis of the phenomena, by application of the temperature influence to different points in the life-cycle, to one or the other sex, in varying degrees, etc.

The account of the present series of experiments will at first deal chiefly with the earlier stages of the development of that method of attack, involving "balanced lethals," which later culminated in the final experiment indicating the effect of temperature. The earlier work on this method was largely independent of other work, but, concomitantly with it, as well as slightly before and after it, another method was being used and improved, involving the X-chromosome. The results with the latter finally exerted an important influence in forcing modifications on the method first referred to. The account of the balanced lethal work will therefore be interrupted, after the first experiment involving it has been presented, and the parallel investigations on the X will be reviewed. These will be followed, finally, by a description of the latest balanced lethal work, in which that method in modified form was carried through to an apparently decisive result.

THE METHOD OF ACCUMULATING MUTANT GENES BY THE AID OF "BALANCED LETHALS," AS AT FIRST APPLIED.

TESTING FOR LETHALS

There is one very serious obstacle in the way of using lethal mutations rather than "visible" mutations as an index of the mutation rate: this is the greater difficulty involved in detecting them. To understand this, it must be recalled that the great majority of mutant genes with which we can deal are recessive, and that, when a mutant gene is first inherited, it usually occurs in only a single individual, in heterozygous condition. If the mutant gene is in an autosome, before it can become homozygous and in any way recognizable, F_2 descendants must be obtained (if we are dealing with bisexual organisms) from this single heterozygous individual. These F_2 must be derived by the inbreeding of just those F_1 which received the gene in question; only one fourth of the pairs of F_1, if mating were random, would have both their members of the required composition. In the F_2 from these particular F_1 any "visi-

ble" mutation could then be detected (in the ratio 1:3); if, however, the gene were lethal, the experimenter would become aware of its existence only if it happened to be in the identical chromosome with, and linked to, another heterozygous recessive "visible" gene, that was previously expected to occur in the same F_2 in the 1:3 ratio. The absence of some or all of the expected "visible" recessives (causing a departure from the 1:3 ratio expected) would here point to the existence of the lethal, and further tests might be made to confirm this conclusion. In the case of autosomal genes, then, the number of individuals that can be used in a count of the frequency of mutation is limited to the number that can be bred, in a special way, as *grandparents*, and, even so, when lethal or other than visible mutations are being looked for, only those lethals in particular chromosome regions, in which a preordained genetic arrangement was present, can be found.

The case is different with mutant genes in the X-chromosome, for here the males allow all visible mutant genes which they received to be apparent in them by mere inspection; lethals in the X would kill the males directly, however, and so would escape detection. The lethals could only be found by breeding females containing them; they would then kill half of the sons of these females, resulting in a 2:1 sex ratio (in which certain expected classes of the males would be absent or diminished in numbers, if the female had also been heterozygous for known visible genes in the X-chromosomes). Thus the existence of such lethals could not be recognized until F_1, and the number of individuals available for a "mutation count" will, in the case of sex-linked lethals, be limited to the number of *mothers* whose offspring can be examined, whereas in the case of sex-linked visible mutants the count consists of the total number of *male offspring* examined. In *Drosophila*, for the same expenditure of time and labor, the latter count will ordinarily be 50 or 100 times as great as the former (since one generally obtains 50 to 100 sons from each mother bred), and it will be still more in excess of the grandparental count required in the case of autosomal mutants. So far as ease of detection is concerned, then, sex-linked visible mutant genes present incomparably the most favorable material, sex-linked lethals being very far inferior to them in this respect, autosomal visible mutants still worse and autosomal lethals standing decidedly at the bottom of the list.

Thus we see that, although exceedingly large counts seem called for in the mutation work, the counts to be obtained, when lethals are looked for, are limited to the number of grandmothers, or, in the case of sex-linked lethals, to the number of mothers, bred, instead of consisting of

all the offspring inspected, as they do in most experiments on crossing over, non-disjunction, etc. The rate of counting may thereby be reduced to a value perhaps 1/100, or even 1/1000 of the rate in ordinary experiments. This difficulty might seem largely to nullify the advantage attaching to the relatively high frequency of origin of lethals, unless a method could be developed which would radically increase the number of individuals available for a count of lethal mutations, with a given expenditure of time and labor.

THE INCREASE IN NUMBER OF MUTANT GENES IN SUCCEEDING GENERATIONS

In developing such a method, advantage was taken of the fact that if a given lot of individuals, known to contain no mutant genes at the start, is bred through a series of n generations (that is, to "F_n"), and one of the individuals of this last (n^{th}) generation is then tested for mutant genes (by obtaining F_2 from it in the way above described, if autosomal genes are in question), this test will reveal all mutant genes that arose in any of the preceding n generations, in the chromatin ancestral to that which eventually entered the individual in question and was subjected to the test. The ancestral chromatin amounted to a total equalling that of at least one gamete, in each of the n generations; hence the single test, carried out after n generations, really informs us of the number of mutations which occurred (in the chromosome regions investigated) in n gametes, and it is equivalent to n ordinary tests. In this way the value of the tests, the "count" which a certain number of tests represents, may be multiplied n-fold,—according to the number (n) of generations through which the experimenter finds it feasible to carry his cultures and accumulate his mutant genes before making the tests. It is true that the mere raising of the cultures in each generation requires labor in itself, but this is routine, and very small compared with that which would be necessary in the special crosses and examinations involved in the same number of tests.

If this were all there were to our problem, its solution would be comparatively simple, merely requiring that the cultures be carried through a large number of generations before being tested. This by itself, however, would not give us a true picture of the average frequency of mutation, because the chromatin that survives to be tested, either in nature or in ordinary experimental cultures, is selected material. That is, random breeding, and more especially the close breeding of most small experimental cultures, may result in a mutant gene becoming homozygous

several generations after its origin; if it is a lethal, it will then eliminate itself, and even if it is "visible," but causes a reduction in viability, it will have a greater tendency to become eliminated than the chromatin in unmutated individuals. The individuals existing after *n* generations, therefore, will come from a selected line of ancestors, in which, on the average, fewer than the usual number of mutations had occurred—how many fewer could not be calculated accurately. Our next task, therefore, must be to stop the process of "natural selection" (as it occurs in ordinary material).

THE PREVENTION OF DIFFERENTIAL SURVIVAL

This end was accomplished by the expedient of artificially constructing a stock containing "balanced lethals." In such a stock, both homologous chromosomes of a certain pair are, in all individuals alike, equally and maximally handicapped for the selection process to start with, by having a lethal gene already inserted into them. Thus, if another lethal arises in these chromosomes later, by mutation, this new lethal cannot put the chromosomes to any furthur disadvantage in selection.

The lethals intentionally inserted into the two given homologous chromosomes of each individual are different ones, lying at different loci, otherwise the individual would be a homozygous lethal and could not live; since, however, the two lethals are in different loci, each of the two chromosomes in question in the individual contains, at the locus where the other chromosome contains a lethal, a normal gene which is dominant to that lethal and so saves the individual's life. Neither of these lethal-bearing chromosomes is at a selectional disadvantage, *as compared with the other*, since both are lethal and neither could ever survive in homozygous condition. There are, however, no normal chromosomes to compete with them so long as individuals of such a stock are bred together exclusively. Such interbreeding of any two individuals of the doubly heterozygous stock must result in the formation of zygotes in the ratio:1 homozygous for one of the lethals (this will die) :2 heterozygous like the parents (these will live) :1 homozygous for the other lethal (this too dies). The heterozygous balanced lethal condition hence automatically perpetuates itself. To be sure, if there were crossing over between the lethals some crossover homologous chromosomes, having neither lethal or both together, would be formed, and this would upset the "balance," but crossingover can be prevented or hindered by the employment of special factors, or rather, genetic conditions, hitherto called "*C* factors," in one of the chromosomes, which interfere with its exchanging parts with its homologue.

This balanced lethal condition will prevent natural selection from tending to eliminate chromosomes (of the pair in question) in which further recessive mutations—lethal or merely deleterious—occur, because these chromosomes never have the opportunity to appear homozygously anyway, on account of the lethals in them from the start, and so the new mutant gene can never exert its deleterious influence. A mutated chromosome will, in other words, have as good a chance of surviving as will its non-mutated sister chromosome, present in a sister individual, since both alike can only survive if heterozygous, and then can survive equally well. The chromosome, being already *maximally* "handicapped" by the original lethal in it, cannot have its survival value decreased any further by acquiring another lethal, and "natural selection" is thus prevented.

The idea of making use of balanced lethals for this purpose readily suggested itself, as a result of the writer's previous investigation of the case of "beaded wings,"—in which he found that such a condition had arisen "automatically," in the course of a selection for beaded that occurred before the stock came into his hands.

THE SYNTHETIC BALANCED STOCK

The writer chose the second chromosome of *Drosophila melanogaster* for the artificial balanced lethal arrangement because, at the time the work was started (1918) this was the only chromosome in which "*C* factors" were known that would prevent crossing over throughout most of the extent of a chromosome. The individuals of the stock first used were provided, in one of the members of their "second" pair of chromosomes, with the "*C* factors" termed "$C_{II\ L}$" and "$C_{II\ R}$" (found by STURTEVANT), the first of which prevents, when heterozygous, practically all crossing over in the "left" half, and the second, nearly all crossing over in the "right" half of this pair; in the region in the middle there is, however, a small amount of crossing over. With each of these "*C* factors" there was also associated a lethal factor, practically inseparably linked to it ($l_{II\ L}$ and $l_{II\ R}$, respectively): these furnished the initial lethal effect for one of the chromosomes. The recessive mutant genes for "plexus" venation (p_x) and for "speck" on thorax (s_p) also happened to lie in this same chromosome, at the right end. As they did no harm, and would have been difficult to remove on account of the hindrance to crossing over, they were allowed to remain.

The homologous chromosome was furnished with the gene for "star eye" (*S*) at the "left" end, as its lethal; this has a dominant visible effect upon the eye but the lethal effect is recessive. In addition, since

there was some crossing over in the middle, this chromosome was provided, at its "right" end, with the gene for "morula eye" (m_r), which, though not lethal, causes sterility in the female and hence is somewhat similar to a lethal in propagative value. Between these two terminal genes star and morula, five recessive genes for visible characters, in loci scattered rather far apart, were included: these were the genes for "vortex" on thorax (T^v), "black" body (b), "purple" eye (p_r), "curved" wing (c), and "arc" wing (a_r). They were inserted into the chromosome so that, when the tests for lethals were finally made—in stock in which crossing over was again allowed, to get rid of the lethal, star, as will be explained—the existence of any new lethal that had arisen by mutation in this chromosome could easily be recognized, by reason of the non-manifestation, in F_2 of the test crosses, of the genes to which this lethal was most closely linked. The locus of the lethal could be approximately determined at the same time by noting the relative frequencies of appearance of the recessive characters dependent on these different loci. Unfortunately the gene for vortex, which was the only mutant gene then available in the chromosome region in which it lies, requires a recessive intensifier ($v_{x\ \mathrm{III}}$), located in the third chromosome, to allow it to show regularly when homozygous; the stock had therefore to be supplied with this intensifier.

As finally made up, then, the balanced lethal stock first used had the following composition, representing the genes in each of the homologous chromosomes concerned on separate lines:

$$\frac{S \quad T^v \qquad b\ p_r\ c \qquad a_r \qquad m_r}{l_{\mathrm{II\,L}} \quad C_{\mathrm{II\,L}} \qquad C_{\mathrm{II}\,R} \quad l_{\mathrm{II}\,R} \quad p_x \quad s_p} \qquad \frac{v_{x\,\mathrm{III}}}{v_{x\,\mathrm{III}}}$$

It will be realized that the construction of this stock, started in the fall of 1918, required nearly an academic year, since few of the adjacent genes of the upper chromosome were already connected together, and the addition of $v_{x\ \mathrm{III}}$ also presented complications. The work with the latter gene had to be carried on "in the dark," as it were, inasmuch as neither of the genes, T^v or $v_{x\ \mathrm{III}}$, can manifest themselves unless both of them are homozygous simultaneously, and, in the stock to be made up, T^v had to be heterozygous.

Later in the year, the easily recognizable recessive character "dumpy" wings arose in the Drosophila laboratory as a mutation, and the present writer found, in analyzing it, that it was allelomorphic to, that is, in the same locus as, vortex (T^v); its gene may be designated as T^d. There was time to insert it in place of the less readily detectable gene for vortex in

some of the cultures of the experiment; and in these cultures v_x III was not added, since dumpy requires no intensifier for its manifestation; otherwise, however, the stock containing dumpy was constructed like the other stock.

THE MAINTENANCE OF SEPARATE LINES OF DESCENT

It might be imagined that all that was now necessary was to raise several large mass cultures of these balanced stocks, carry them through a considerable number of generations ("n") and then apply the breeding tests for lethal mutations to a large number of individuals from each culture. Such a technique would, however, be inadequate, for the different tested individuals from a single culture might be more or less closely related; we should have no way of knowing in what generation the lines of ancestry of their tested chromosomes converged, and consequently we could not tell how many ancestral chromosomes the tests really applied to. In other words, common ancestors should not be reckoned more than once in the accounting, but in mass cultures it cannot be determined during how many generations of the ancestral cultures two given individuals have had a common ancestor. There is little or nothing to be gained, therefore, in testing more than one individual from each culture, even though the latter is a large mass culture.

It follows from the above that in order to be able eventually to test a large number of individuals all of which have lines of descent that are known to have remained separate since the beginning of the experiment, it is necessary to maintain in each generation an equally large number of separate cultures, or "lines" of descent, to carry each of these lines through the series of n generations separately, without allowing inter-breeding between it and the other lines, and finally to take just one sample individual from each line, for the crosses which are to give the test for mutant genes. The separate cultures, with this method of breeding, may be reared in small containers (vials), only large enough to prevent too great risk of the lines becoming extinct.

If, now, we have l separate lines which have been bred, on the average, through n generations, and we then test one individual from each line, our l tests will, according to the mode of reckoning previously explained, inform us of the number of mutations that occurred (in the chromosome loci tested) in ln individuals. The size of the "mutation count," then, is the product of the number of lines tested by the number of generations.

THE RECOGNITION OF ANTECEDENT MUTATIONS

From our total number of mutant genes, found among these *ln* individuals, it is of course necessary to exclude any lethals or other mutant genes that arose in the germ plasm before the specified *n* generations of breeding began. This means either that we must, at the start of the *n* generations of breeding, make preliminary tests of that portion of the chromatin of each line which will be ancestral to the chromatin finally tested in that line (this would double the total number of tests necessary) or that we must devise some method of distinguishing, in our final tests, between those mutant genes that were originally present, and those that arose in the course of the *n* generations of breeding. The latter object can be achieved by having the lines related in groups, in some known definite way, at the start of the experiment.

The "sister lines" of a given group, having each of their chromosomes (of the type to be tested) immediately derived from a common ancestral chromosome in the common ancestor of the group, will share any lethals (or other mutant genes) which may unintentionally have been present in that common ancestral chromosome; if such were present, then, the sister lines will contain identical lethals. The identity of these lethals, once they have been discovered in the final tests, can easily be established by crossing them together and determining whether the combination effect is lethal also. On the other hand, those lethals which were not present at the start, but have arisen independently, after the lines of a group were split off from their common ancestor and the experiment proper began, should not coexist as identical lethals in all the lines of the group.[4] Such lethals only will be given a place in our "mutation count."

INSURING THE UNITY OF THE SOURCE OF TESTED CHROMATIN IN A GIVEN GROUP OF LINES

In making the final tests, we must be certain that the chromatin tested in a given line is really descended from the supposed ancestral chromatin

[4] It is true that BAUR's work on "premutation," and the gene-element conception, indicate that identical mutations may really be expected more often in related than in non-related lines, but in the actual data from our first mutation experiment with the balanced lethal stock, where there were usually 4 to 8 lines in a group, a given mutant gene appeared either in all the most closely related lines of the group or in only one line, never in part of the lines in such a way as "parallel mutation" would have produced. Hence such a tendency was not strong enough, in this work, to have caused an appreciable proportion of the mutations that occurred after the splitting of the lines to be reckoned as having occurred beforehand, and the above method remained valid here. But even if there were a strong tendency to "parallel mutations" in related lines ("premutation") it would still be valid to exclude these, when they could be recognized, and compare the residual numbers of "independent mutations" in different series of lines to determine the effect of the given agencies upon the rate of occurrence of these mutations.

which was either directly tested, by preliminary tests, or, as above stated, tested later by means of the final tests of the other "sister" lines of the group. This condition requires all the flies of a culture to have their chromatin (of the type for testing) derived from a single ancestral source—a result which could not be achieved by using a pair of flies of similar composition to start the group of lines, for in that case some of the flies of later generations might derive the chromatin in question from the original male, and others from the original female. It is accordingly necessary to know that a single member of the original pair, which member may be called the "source" individual, has supplied the chromatin (of the type in question) to the later generations of the sister lines of a group. Furthermore, since this source individual must be diploid, we must likewise know that all the chromatin in question was derived from the same genetic half of that source individual.

These purposes can be carried out by using as the source a single heterozygous male (preferable to a female for this purpose since in the male there can be no crossing over whatever), in which the chromosome in question is distinguished from its homologue by a "visible" gene or "identifying factor" (ALTENBURG and MULLER 1920) that will show in the next generation. This male is crossed to a female which does not contain a chromosome like the "source chromosome" just referred to, but contains the chromosome which is to be the partner of the former in the balanced stock. This "partner chromosome," when in this female, must exist in heterozygous condition, since it is necessary for it to contain a "balancing" lethal; the other homologous chromosome of the female, which is not to be used later, must then be marked off from the "partner chromosome" by another "identifying factor." When the cross between such a male and female is made, the offspring of the required balanced-lethal type can be distinguished from the rest by their "identifying factors," and used to form the start of the sister lines; they all necessarily derive their chromatin of the type to be tested from an identical source.

In the first mutation experiment involving the balanced lethal lines, the groups were started by using pairs (or one male and several females per culture) of the following composition:

$$\frac{ST^{v}b\ \ p_{r}c\ \ a_{r}\ \ m_{r}}{l_{\mathrm{II\,L}}C_{\mathrm{II\,L}}C_{\mathrm{II\,R}}l_{\mathrm{II\,R}}\,p_{x}s_{p}}\ \frac{v_{x\mathrm{III}}}{v_{x\mathrm{III}}}\ ♂ \times \frac{l_{\mathrm{II\,L}}C_{\mathrm{II\,L}}C_{\mathrm{II\,R}}l_{\mathrm{II\,R}}\,p_{x}s_{p}}{b\ p_{r}\ \ c\ \ \ p_{x}s_{p}}\ \frac{v_{x\mathrm{III}}}{v_{x\mathrm{III}}}\ ♀$$

Here the genes S, p_x, and s_p were the "identifying factors" in the source male, and b, p_r, and c were the "identifying factors" in the females. Of the 4 possible zygote combinations formed in this cross, it will be seen that only

one class of offspring appeared star, but otherwise normal; these were the offspring having the required balanced lethal composition, and, in any one culture, all the offspring of this type derived their chromatin of the kind to be tested from one source, namely, from one genetic half of the one male parent. Such offspring were picked out, and bred together in mass culture—one mass culture from the offspring of each original male—to form the first "sister" line of the group of lines from that male.

The second "sister" line of a given group was formed, in each case, by taking a second single male from among these offspring, that is, a brother of the mass culture used to form the first sister line, and crossing it to females like those first used

$$\text{(namely, } \frac{l_{\text{IIL}}\, C_{\text{IIL}}\, C_{\text{IIR}}\, l_{\text{IIR}}\, p_x s_p}{b \quad p_r \quad c \quad p_x s_p} \; \frac{v_{x\text{III}}}{v_{x\text{III}}} \text{)}.$$

As the second male had a composition like that of the source male of the group it in turn produced offspring from which, by selection of the star, but otherwise normal appearing, flies, a second mass culture (the second sister line) could be started, and from which likewise a single male could again be isolated for crossing as before, in order to establish the third and eventually still further lines. So the process was continued until, in some of the groups of the series containing vortex, 9 "sister" lines had been established. It will be noted that the lines thus termed "sisters" are not sisters in the ordinary sense of the term, but really stand in direct descent, one from the other. This particular type of relationship allowed of the test of the frequency of "parallel" mutations, referred to in the previous footnote.

Although preliminary testing of the lines was avoided by having them grouped in this way, considerable labor was nevertheless involved in making all the crosses and selections (especially the selections of virgin flies) necessary in getting the lines started. The establishment of the lines of the "vortex series" was begun in May and continued until November, 1919, nine generations later. At that time the stock containing dumpy in place of vortex was ready, and the establishment of these lines was begun, by means of the same kind of procedure, and carried on through six successive generations. In all, 94 vortex-containing and 249 dumpy-containing lines were started.

THE TESTING OF THE BALANCED LINES

To complete our preliminary account of the balanced lethal method, there now remains to be described the procedure necessary for making

the final test of the sample individual from each line. In order to become aware of what recessive lethals or other mutant genes the chromatin in question, of a given fly, contains, it is of course necessary to give that particular chromatin an opportunity to become homozygous. This cannot be accomplished by simply mating together two individuals of the same culture and thus allowing this chromatin to come into combination with the corresponding chromatin of the other individual, because, firstly, that corresponding chromatin may not be identical with the first and so may not contain the same mutant genes (owing to mutation having occurred since the two branched off from their common ancestral chromatin), and because, secondly, the lethals that had been intentionally inserted to preserve the "balance" would prevent the homozygotes from appearing anyway. A single "sample fly" must therefore be taken from each line of the experiment (in the "F_n" generation), and outcrossed with a fly from a different stock, that does not contain a chromosome similar to that being tested. Those F_1 or rather, F_{n+1}, flies from this cross which show, by their "identifying characters," that they have received from their heterozygous parent of the experimental line the chromatin which is to be tested, are then crossed together, to allow an opportunity for F_{n+2} individuals homozygous for (portions of) this chromatin to be formed.

In this latter cross, there must be a means of preventing the original "balancing" lethal—in our case, star—from killing all the F_{n+2} flies that might be homozygous for any of the chromatin in question; otherwise the effect of any new lethal or other new mutant gene in this chromatin would be obliterated. Such obscuring action of the balancing lethal will be prevented naturally, in some of the F_{n+2} flies, if crossing over between this lethal and the rest of the chromosome is allowed in F_{n+1}, for some of the crossover F_{n+2} flies will then receive from one of their parents (the F_{n+1} female) part of the chromosome to be tested, without this lethal being attached to it, and from their other parent (the F_{n+1} male) the entire chromosome to be tested. The original lethal, being only heterozygous in such an F_{n+2} fly, will then fail to kill it, but the fly will be homozygous for part of the rest of the chromatin to be tested. Which part is thus homozygous will be revealed by the manifestation of the recessive visible genes previously placed there (in our present case, T^v, b, p_r, c, a_r, and m_r). If, now, the homozygous chromatin of the given region contains a visible mutant gene that arose in the course of the experiment, this gene may be seen to produce its characteristic effect in the fly (barring interference between its manifestation and that of the previously provided visible mutant genes), but if this homozygous chro-

matin contains a newly arisen lethal the fly containing it will die even though it is not homozygous for the original lethal (star). Since all such flies in the given culture will be killed, the existence of this lethal can therefore be inferred by the very absence of a certain class or classes of crossover flies that would otherwise be expected to appear, and the locus of the lethal can be estimated by noting just which classes of crossover flies are absent, and to what extent other classes are reduced in numbers.

In applying this method in the present instance a single fly—a male, to avoid all crossing over here—from the "nth" generation of each culture, was mated to a female not containing $C_{II\ L}$. The latter is the "factor" which, when heterozygous, prevents crossing over in the left half of the chromosome. As this half is the region containing the locus of the original lethal, star, which it is intended now to get rid of, by crossing over, it will be seen that in the offspring (F_{n+1}) of this female the desired crossing over can now take place. The female was, however, provided with the combination $C_{II\ R}\ l_{II\ R}\ p_x\ s_p$, in order that the determination of new lethals in the right half of the chromosome might not be obscured by too much crossing over. She was only heterozygous for this combination, since it is itself lethal, and she was provided in her homologous second chromosome with the "identifying factors" arc and morula ($a_r\ m_r$). In addition, she had been made up to contain, in homozygous condition, the vortex intensifier, $v_{x\ III}$. Her composition was therefore as follows:

$$\frac{C_{II\,R}\ l_{II\,R}\ p_x\ s_p \qquad v_{x\,III}}{a_r\ m_r \qquad v_{x\,III}}$$

It will be seen that when such a female is crossed to a male from one of the vortex-containing experimental lines, one quarter of the (F_{n+1}) zygotes formed will have the desired composition, namely:

$$\frac{S\ \ T^v\ \ b\ \ p_r\ \ c\ \ a_r\ \ m_r \qquad v_{x\,III}}{C_{II\,R}\ l_{II\,R}\ \ p_x\ \ s_p \qquad v_{x\,III}}$$

These will be distinguishable from the others by having star eyes and being otherwise normal. They contain the test-chromosome, but not a chromosome that prevents crossing over between star and the other genes. In the case of the dumpy-containing cultures, a cross to the same kind of female was made, although here the presence of $v_{x\ III}$ was not necessary.

The star F_{n+1} males and females from each culture were then crossed *inter se*, virgin females being used, and the F_{n+2} of each cross were ex-

amined to see whether all parts of the "test-chromosome," except the star-containing region to the left of T^v or T^d, manifested themselves in some flies or other, in the homozygous condition. That is, the observer had to make sure that there were at least a few flies, in the culture, that combined the characters of T^v (or T^d) and b (these were homozygous for the region T^v-b), some that combined b and p_r, some that combined p_r and c, and some c, a_r, and m_r (which practically never separated because of the $C_{II\ R}$). It was also noted whether or not any of these combinations that appeared showed any new visible mutant characters.

The above determination required the obtaining of a considerable number of flies in the F_{n+2} cultures, since only about 8 percent of cross-overs occur between the S and the T locus, and of these 8 percent only one fourth would form recessive homozygotes, and they would have a relatively low viability. There were many cultures, therefore, in which flies of the T^v-b combination failed to appear, even in cases in which there was no lethal in this region, just because of the relatively large "error of sampling" that applies to such small expected numbers. All the cultures that gave such results were therefore regarded as "doubtful cases" at first, that had to be followed up, in later generations, to make sure whether or not a lethal were really present. This following-up process was especially cumbersome, as flies of the requisite composition for continuing the study could not be recognized with certainty in the F_{n+2} population. The same difficulty applied in each generation in which the lethal and the "doubtful" cultures were continued for more exact locus determination. In the locus determinations of lethals that proved to be in the right half of the chromosome outcrosses had later to be made to remove the chromosome containing $C_{II\ R}$, since the latter, by preventing crossing over in this region, made the mapping of the lethal impossible.

Finally, after rather extended special crosses, the detailing of which would take us too far afield, balanced stocks were again synthesized, in the case of all "test-chromosomes" containing a newly discovered lethal. These stocks would maintain this lethal automatically in heterozygous condition, without crossing over, and without further selection being necessary. In these stocks, the original lethal, star, had been removed by previous crossing over, and so outcrosses of these made a more unhampered study of the new lethal possible. Thus, when these stocks were crossed with one another, recessives showing the combination T^v (or T^d) $b\ p_r\ c\ a_r\ m_r$ could appear in one fourth of all their F_1 offspring—*except* where the lethals in the two stocks happened to be in identical

loci. All possible crosses of this type, between stocks having lethals located in the same general chromosome region, were then made, in order to find out just which lethals did lie in identical loci (that is, were "allelomorphs").

It will be noted that, in the above work, only the $S\ T^v$ (or T^d) $b\ p_r\ c\ a_r\ m_r$ chromosome of the balanced lines was investigated for new mutant genes. The other chromosome—containing $l_{II\ L}\ C_{II\ L}\ C_{II\ R}\ l_{II\ R}\ p_x\ s_p$—could not be subjected to testing, because the "*C* factors" here prevented this chromosome from getting rid of the "balancing" lethals $l_{II\ L}$ and $l_{II\ R}$, that were present in it from the beginning, and so the existence of no new lethal recessive mutant genes in that chromosome could be ascertained.

CONDITIONS OF REARING OF THE LINES IN THE FIRST EXPERIMENT UTILIZING BALANCED LETHALS

The 343 lines, which were to be subjected to the "*n*" generations of breeding, followed by testing of the sort above described, were intended mainly to provide a preliminary series of "control" results that would give an estimate of the frequency of mutation in the second chromosome under certain definite or "standard" conditions, which could readily be reproduced, and which would *a priori* be likely to give a relatively high mutation rate. It was planned later to run other experiments in which the lines were to be bred under other conditions, after the "control" results had been obtained, and then to compare the special with the control results in order to determine the effect on mutation rate of the special methods of breeding or treatment later used. For this reason most of the lines in the preliminary experiments were carried on in one standardized fashion, which involved keeping them in 4 by 1 inch vials, in an incubator at a temperature of 26.5°C. It should be stated, however, that previous to November 7, 1919, when only the vortex series was being established or propagated, the cultures were kept at room temperature. After that, the vortex lines and most of the dumpy lines were placed in the incubator, where they were able to develop at approximately the maximum rate. In the incubator the temperature seldom varied more than 1°C, except for short periods in the summer of 1920, when the room temperature (in COLUMBIA UNIVERSITY, New York City) became higher. The food used consisted of freshly prepared 1 percent agar in 5 parts water, 2 parts mashed banana, and 1 part karo syrup, sprayed with a suspension of yeast, after gelling, and sprinkled with white confetti.

One week after the parents were placed in a culture at 26.5°C, they were thrown out, and two week later the offspring (a sufficient number of which had then hatched) were tansferred directly, *without etherization*, to a fresh culture vial, in which the above cycle was repeated. These fortnightly transfers were carried on for approximately a year (more in the case of lines that had been established earlier, less in those established later). This work of mere propagation was for the most part performed by a supervised assistant, although of course all the crosses necessary for synthesizing the stocks, establishing the lines, testing them, and investigating the lethals, were carried out by the writer. The fact that very few lines at the end showed the effects of contamination indicated that the work of propagation had been carried on with due care, for the germ plasm of a fly without lethals, that had entered a culture, would tend rapidly to upset the special mechanism of "balancing," and to supplant the lethal-bearing germ plasm, by a process of natural selection.

The opportunity could not be resisted, however, of carrying on a minority of the cultures under a different condition, in order that some idea might be obtained of the controllability of the mutation rate in the second chromosome, before too many years elapsed. Temperature was the condition which it was chosen to vary, for the reasons previously given, and because some suggestive results had already been obtained with it on the X-chromosome in the summer of 1919, as will be explained. As there were not facilities for securing a constant temperature much below 26.5°C, and as *Drosophila* does not withstand much higher temperature, the writer contented himself with making the difference in this experiment one of sign rather than of fixed quantity. The vials containing the "cooler" lines were hence kept at the room temperature of COLUMBIA UNIVERSITY, in the cold weather, and in the warm weather they were kept in dishes covered with wet cloths connected with trays of shallow water; over these wet dishes an electric fan was usually kept playing. In this way, the cooler vials were kept about 8°C cooler than the others, on the average, throughout the year. This difference in temperature was reflected in a slower rate of development, and in the case of these vials it was feasible to make transfers, on the average, only once a month. One hundred and thirteen of the lines, all belonging to the dumpy-containing stock, were reared under the cooler conditions. The cooling treatment was started November 7, 1919, when the first lines of this series were established, at the same time as the incubator treatment of the other lines (94 vortex and 136 dumpy) was instituted.

Before the differential temperature treatment in this first balanced lethal experiment had been begun, and during the time that the vortex-containing lines were being synthesized, established and propagated, certain mutation experiments on the X-chromosome were being initiated, in which it was not possible to use the method of balanced lethals. The results of these were obtained prior to those from the fore-going experiments, and, as has been mentioned, they had an important bearing upon the conduct of the later experiments on balanced lethal lines. However, to preserve the consecutiveness of the present account, the results of the balanced lethal experiment which we have been describing will be presented before those of the X-chromosome work.

RESULTS—DETERMINATION OF AN AUTOSOMAL MUTATION RATE, AND OF A SIGNIFICANT VARIATION IN THE TIME-RATE OF MUTATION ASSOCIATED WITH THE TEMPERATURE DIFFERENCE

The balanced lethal lines, started at COLUMBIA UNIVERSITY and at WOODS HOLE in the spring, summer, and fall of 1919, in the manner above described, were tested, by the methods explained above (pp. 296-9) in the fall and winter of 1920-21, after they had been moved to the UNIVERSITY OF TEXAS. When the total number of "chromosome-generations" (the product $l\,n$) in each series had been determined (in practice, by adding the numbers of generations in the individual lines, since not all lines were kept for the same length of time), it was found that in the vortex-containing group (all kept at 26.5°C for the last 11 months, comprising 7/8 of the generations) there was a total of 1918 tested individuals, or "chromosome-generations" (obtained by testing the 73 lines surviving out of the original 94); in the dumpy-containing "warmer" group (also at 26.5°C, throughout the experiment) there were 2180 chromosome-generations" (from the 106 lines surviving out of the initial 136), and in the dumpy-containing "cooler series" 726 chromosome generations (from the 71 lines left out of 113).

In the vortex lot there were 8 new lethals and in the dumpy-containing lot that was also kept at the warmer temperature there were 16. No visible mutations were detected. In other to determine whether the difference between the mutation rates in these two lots is "significant" we may apply the usual formula for the probable error of a difference between small proportions: that is, $0.6745\sqrt{\frac{P}{n_1 n_2}}$. P in this case is the absolute number of mutations in the two lots taken together, namely

24, n_1 is the total count of tested chromosome-generations in one of the lots, namely 1918, and n_2 that in the other lot, namely 2180. Substituting these numbers in the formula, we find that the difference in rate here is only twice its own probable error, and hence practically without significance by itself (chance 1 in 5),—despite the fact that the stocks were not genetically identical. It may therefore be legitimate, for some purposes of comparison at least, to average the two counts together; we then obtain a mutation rate of 1 lethal in about 170 chromosome-generations, or 0.58 percent, for the second chromosome, in these combined warmer lots.

It has been explained that this was not the first figure ever obtained for mutuation rate in any chromosome, as some of the results of the X-chromosome work, which will be reviewed later, were obtained previously, but it was the first figure obtained for an autosome. As will be seen, it was of the same general "order of magnitude" as most of the figures obtained with the X, although, on account of the great variations found in the latter, exact comparisons with them would be of little value. This "order of magnitude" of the lethal mutation rate is higher than that which had been expected. It implies a rather rapid deterioration of the germ plasm when protected against natural selection, for 1 lethal per 170 chromosome-generations, when the generations occupy two weeks, means that, on the average, there will be one lethal to each unselected second chromosome after the passage of six and a half years, two lethals per chromosome after thirteen years, etc. In terms of individual genes, of course, the rate is much slower. If we consider each long autosome as containing at the very least 600 genes (a revised figure based on calculations given by the writer in 1926), then each of the contained genes will, on the average, give a lethal or otherwise detectable mutation not oftener than every 3,900 years, provided this rate of mutation continues.

In the much smaller "cooler series" only 2 lethals were found—a rate of only 1 in 363, or 0.27 percent. This count in the cooler series is so small that the difference in rate of mutation, per chromosome-generation, between all the warmer and the cooler lines turns out to be only 1.6 times its probable error, when we apply the formula given above. This result, then, "lacks significance," if taken by itself, as the chance of obtaining as great a difference, in either direction, if the rates were really equal, is as high as 1 in 3.3. It will be noted, however, that the difference is in the same direction as in the temperature experiment on the X-chromosome, and in the same direction as most heat effects; the chance

of obtaining such a difference in this direction is of course only half as great.

It has been stated that the lines kept at the lower temperature took twice as long to develop as the others,—the length of time necessary for their cultures to produce a sufficient number of flies for transferring being four weeks, as contrasted with two weeks in the other series. If mutation occurs at a fixed time-rate, regardless both of the temperature (and the consequent speed of metabolism), and also of the stage in the life cycle, and the cell cycle, in which the genes exist, the cooler lines which had passed through 10 generations should tend to produce the same number of lethals as an equal number of warmer lines which had passed through 20 generations in the same length of time. In that case, it would be more legitimate to measure the mutation rate in terms of units that may be called "chromosome-months," rather than in "chromosome-generations." When this method of measurement is employed, the tests on the cooler lines are found to make twice as respectable a showing as before, in total "units" counted, relatively to the tests on the warmer lines. In accordance with this relation, the difference between the mutation rates of the two series, per "chromosome-month," is much greater than that per "chromosome-generation." The time-rate of mutation, in this sense, is 1.17 percent in the warmer cultures and 0.31 percent in the cooler and the difference, 0.86 percent, is subject to a probable error due to random sampling of 0.3 percent. Thus the difference in the time-rates is 2.9 times its own probable error—a "chance" of only 1 in 18.5, regardless of direction, or 1 in 37, if we consider the direction of the change as specified. If we confine our reckoning to the "dumpy"-containing cultures exclusively, we find that the difference in time-rate of mutation between the warmer and the cooler sets is 3.7 times its probable error, a result that would ordinarily be regarded as convincingly positive.

The data therefore seem to yield fairly good evidence that the assumptions on which the latter calculations were based are, one or more of them, incorrect: that is, we are led to conclude that probably the time-rate of mutation is not independent both of temperature, of the speed of "vital activities," and of the stage in the life (or cell) cycle, and that therefore temperature, whether or not it effects mutation frequency directly, can at least effect it indirectly, or through its influence on some of these phenomena. It may perhaps be claimed here that such an effect might have been taken for granted before-hand, but it is by making such assumptions gratuitously that biology progresses over-slowly.

Knowing as we do nothing about the mechanism of mutation, we could not be sure in advance that its speed is limited by that of a chemical reaction and that it hence is highly responsive to temperature changes; still less could we presume to say that the reactions of ordinary "metabolism" are the causative agents in it. Neither have we, until the past year at least, had any valid evidence (except in the seemingly special case of variegation in corn), indicating that mutation, in the sense of alteration of the gene, occurs preferentially at any particular stage in the germ track cycle, though there has been a little evidence contrary to this idea (MULLER 1920). Unless such a relation existed, the mere breeding of individuals at an earlier age would not result in the occurrence of more mutations after a given long period of time, for the mutations would simply have gone on occurring with the same frequency as otherwise, regardless of the fact that the maturation period, etc., had been passed through oftener. It is therefore of importance to measure the frequency of mutation, under various conditions, not only in terms of its rate per generation, but also per unit of time. It is in terms of time units, also, that the effect of temperature on chemical reactions in general is usually measured, and so a comparison with the latter may best be made in these terms.

Unfortunately, despite the seemingly plausible evidence from the present experiment that the time-rate of mutation is affected by temperature under the conditions given, judgment on this matter must be withheld for a while. For, as we shall see later, the work which was being done on the X-chromosome at the same time as, and subsequently to, the work now being described, showed that some unknown factors which ordinarily are not controlled in an experiment may cause significant differences in mutation rates. In the present instance, cultural factors other than temperature itself and conditions caused by it (for example, state of the food) could scarcely have accounted for the effect, since the warmer and cooler cultures were run simultaneously, and were subjected to the same conditions, aside from those dependent on temperature. The possible influence of invisible genetic factors could not categorically be excluded, however, as means whereby the difference in mutation rate might have been caused, since the ancestors of the warmer and the cooler dumpy lines, though they looked alike, might have been differentiated in regard to the proportions they carried of alternative allelomorphs that could not be seen. This seems a rather hypothetical objection, perhaps, specially created to make the difficulty, and the essential agreement between the mutation rates in both "warmer" lots,

vortex and dumpy—although these were known to be different genetically,—does not lend it support. It will therefore be necessary to describe the X-chromosome work in some detail, to show that the point cannot be ignored, and, after this, the latest experiment on the second chromosome will be described, wherein care was taken to avoid this objection.

In concluding the account of the present experiment, we may call attention to the details that were found out concerning the lethals that had appeared. All these lethals were subjected to linkage tests for the determination of their loci, in order to make sure that there was nothing grossly anomolous in the distribution of the mutating loci. The results are graphically presented in figure 1. As we shall later note in the case

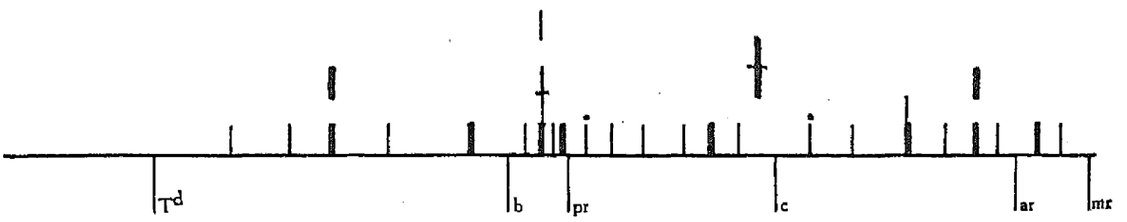


FIGURE 1.—Figure to illustrate mode of distribution of lethals arising in the second chromosome, based on data from the first "balanced lethal" experiment on this chromosome. Genes for visible characters furnishing standards of reference in this mapping are indicated by labelled lines joining from below the horizontal line that represents the chromosome. Genes for lethals are shown by vertical lines above the horizontal line. Lines arranged in vertical order, one above the other, represent genes known to be in identical loci. In the case of each lethal found in the "cooler series" a dot is placed just above the corresponding vertical line. Lethals of the "vortex series" are shown by heavier lines than the others. "Antecedent" lethals found in the course of these tests are shown above, disconnected from the horizontal line. Lethals to the left of T^d could not have been detected. The distribution of the lethals *among* the regions marked off by the "visible" genes shown below is in nearly all cases exact, but their positions *within* their respective "regions" are only approximate.

of the X-chromosome also, the grouping of the lethals here shows characteristics similar to that known for the genes for visible characters in the same chromosome, for, in this case, there is somewhat of a condensation of genes in the central portion of the map, which may be due to a "fore-shortening" of this region. Mutations did occur in all regions, however, and it is evident from the grouping of the lethals that the experiment dealt with the mutation frequency of the collection of genes in the chromosome as a whole, rather than with that of a few selected loci.

As might have been expected, however, mutations did occur in some loci oftener than in others, that is, in a number of cases a given locus mutated more than once (sometimes repeating "antecedent" mutations), and one especially mutable locus gave rise to four separate lethals (in-

cluding one antecedent to the experiment proper). The latter individual gene hence had a mutation rate that borders on the measurable,—about 0.8 percent. Extensive counts were made of the lethal stocks derived from the mutations of the latter gene, but in 6685 chromosome-generations there were no reverse mutations of the mutant back to the normal allelomorph. Had reverse mutation been as frequent as the original type of mutation this lethal could have been used, like variegated corn, for the study of mutation-rate in its own given locus, since the mutations "backward" to non-lethal could have been recognized on mere inspection, by having visible "identifying factors" linked with the lethal. It should be noted that neither in the case of this nor of any of the other loci were we concerned with effects due to asymmetrical crossing over, like the reverse "mutation" of bar eye, since in the present experiment crossing over had been prevented by the "*C* factors."

LESSONS DRAWN FROM MUTATION EXPERIMENTS ON THE X-CHROMOSOME

THE FIRST ATTEMPT TO ESTIMATE THE LETHAL MUTATION RATE

In order to give an understanding of the development of the work on mutation rate it may be explained that the first attempt at an experiment dealing in any way with the conditions of origination of lethals (together with other mutants) consisted of a series of crosses involving sex-linked genes which the writer had his genetics class, at the RICE INSTITUTE, Houston, Texas, carry out cooperatively in the spring of 1918. Each of the 15 students made ten matings, in separate cultures, of pairs of flies of the composition $\underline{w^e vf}$ ♀ and $w^e vf$ ♂. In the formula for the female here, which is heterozygous, the blank spaces below the horizontal line indicate the presence of the normal allelomorphs of the recessive "identifying factors" that are shown in corresponding positions in the formulae of the other chromosomes. The recessive genes w^e, v and f (eosin eye, vermilion eye and forked bristles) lie near the left end, the middle, and the right end, respectively, of the X-chromosome. It will be seen that a lethal occurring in either of the X's of a mother would be evidenced by the absence of all sons carrying, in combination, the two genes lying on either side of the lethal. Among the offspring formed from the cross there would be some females phaenotypically and genetically like the mother and some males like the father (barring mutations), and these could be used for the repetition of the cross in the following generation. In this manner each student was expected to continue each of the ten matings through three generations, by choosing from each culture one pair of flies (the female to be virgin) of composition like the

parents, to continue the "line of descent." Complete classified counts were made of the offspring, so that not only lethals but also conspicuous changes of crossover frequency might be detected.

As no work had yet been done on gene mutation rate it was not known on what scale the work would have to be carried out, but it was hoped that the (theoretically) 300 cultures that were to be raised during the last two generations (after the preliminary test-generation, which was for for the elimination of lethals previously present) might at least give an idea of the order of magnitude of the mutation rate. In addition, it was thought that part of the cultures might as well be utilized for testing out whether or not some one among various agents might be hit upon, which would be able to produce mutations in such high frequency that the effects would be evident on examination of only a few cultures for lethals. With this object in view, each student kept half of his lines as controls and subjected the others, in each of the three generations, to some particular treatment. The same treatment was given to all of the treated cultures of a given person, but each person used a different treatment. The attempt was made to give the maximum treatment practicable, in each case. Among the agents employed were Janus green, methylene blue, lead acetate, alcohol, KNC, low air pressure, high oxygen pressure, heat, cold, and other easily-applied influences that readily suggested themselves as modifiers of vital structures or activities.

The results of the crosses proved negative, in that, after the preliminary "test-generation," no mutations (or very conspicuous changes in crossover frequency) were found at all, with the exception of one lethal that appeared in a control culture. Needless to say, however, not nearly all of the cultures had been properly carried through, and the experiment as a whole, involving the labor of so many inexperienced persons, could not be considered as yielding very critical data. Nevertheless, it did serve as a first try-out of the method, and at least showed that larger and fully reliable numbers would be desirable for establishing even the order of magnitude of the mutation rate; it indicated besides that not even lethal mutations are readily produced *en masse*. Following this experiment, therefore, the writer changed his mode of attack, and began in the fall of 1918, in New York City, synthesizing the elaborate stocks for the balanced-lethal experiment on the second chromosome which has just been described, in the hope of obtaining, this time, much larger numbers that might establish a significant figure for a "control" set of cultures, and possibly for just one "treated" set (the "cooler series") as well.

At about the same time, in the winter and spring of 1918 to 1919 Doctor EDGAR ALTENBURG, at Houston, Texas, was making an attempt to secure significant numbers in a different way, and his attempt was brought to a successful conclusion much sooner.

ALTENBURG'S ESTABLISHMENT OF THE FIRST FIGURE FOR MUTATION RATE—ITS UNEXPECTED MAGNITUDE

In order not to be hampered in the work of obtaining large numbers in a comparatively short time, ALTENBURG used the most direct method possible—that is, that of testing for lethals in the X-chromosome, rather than an autosome (since this requires only one generation instead of two for the test), and, further, determining the presence of these lethals not by a cross involving "identifying factors" but simply by their effect on the sex-ratio. A lethal present in one X of a mother causes the death of half of her sons, and thus results in a 2 ♀ : 1 ♂ ratio in F_1, instead of the usual 1 ♀ : 1 ♂ ratio, no matter what male she is crossed to.

Of course this method suffers from the disadvantage common to all mutation work on the X-chromosome, that each individual which is represented in the "mutation count" has to be tested separately—lethals cannot be accumulated in the X by the balanced lethal method since a male containing a lethal in its X necessarily dies. Furthermore, the counting necessary to determine the sex-ratio takes more time than a mere qualitative determination of the presence of certain classes, which is all that is usually required when identifying factors are involved. And the sex-ratio, when obtained, is subject to a considerable error of sampling, with the resultant occurrence of relatively many "doubtful cases" that have to be followed up in later generations. This following up becomes particularly cumbersome since, in the absence of identifying factors, there is no way of distinguishing by inspection between the offspring that received the questioned lethal and the others, so that a number of the daughters have to be bred in order to be sure of having at least one with the supposed lethal included. On the other hand, owing to the simplicity and directness of the method, it has certain great advantages, in that (1) virgin females are not required in the testing, (2) there is little trouble with poor viability and fertility, (3) results can be obtained from it almost immediately, and there is hence less danger of the experiment collapsing before results are obtained.

The data concerning this experiment have been briefly given in a previous publication (MULLER and ALTENBURG 1919), but they require review here. The flies were divided into about 75 "lines," each of which

was bred through 6 generations, including the preliminary generation in which the female ancestor of the line was tested in order that any lines containing lethals at the start might be eliminated. (Of course no male ancestor could have had a sex-linked lethal.) In each generation usually not more than one female of each line was tested (since it was desired to insure the independent origin of any lethals found), and this test was at the same time the cross that furnished the flies of that line that were to be bred in the next generation. There were 90 females tested in the preliminary generation and 385 in the other five generations combined, each test involving, as above explained, a count of the number of offspring of each sex. Besides this, there were many crosses necessary in the work of following up those cases in which the sex-ratios did not seem decisive, until it could be determined whether or not a lethal were present. Most of the cases which appeared to give a decisive 2:1 ratio were also tested further, and the lethals thus verified. In this way, by reason of the resolution of the critical cases, the final figures acquired considerable precision.

The definitive data were secured before the summer of 1919. They showed that, among the 385 females tested and known to have come from parents that had not received lethals, 13 carried lethals themselves; in none, however, were there any "visible" mutations detected. Since each female carried two X-chromosomes, this meant a rate of lethal mutation of 13 in 770, or 1 lethal in about 60 "X-chromosome-generations"—in other words, nearly 2 percent of mutation.

This figure, the first real figure ever obtained for the rate of gene mutation in a chromosome, was startlingly high—far higher than most Drosophila workers would have anticipated. It showed that (at times at least) the rate of mutation was high enough to permit of its being studied quantitatively by individual tests of the X-chromosome, without accumulation by means of balanced lethals. It seemed logical, therefore, to push further the work upon the X-chromosome, in the hope of securing additional significant results.

JOINT CONFIRMATION OF ALTENBURG'S FIGURE, AND THE SECURING OF A PROBABLY SIGNIFICANT EFFECT OF TEMPERATURE ON THE TIME-RATE OF MUTATION

ALTENBURG and the writer thereupon undertook a joint mutation experiment upon the X-chromosome, in the summer of 1919 (at a time when some of the balanced lethal lines containing vortex had already been established and new ones were being established). Part of the results of this experiment also have previously been abstracted, in the same article

as the sex-ratio tests (and part in Proc. Amer. Soc. Zool. 1920); but they likewise are so related to the present work as to require mention here. The aims of this experiment were, first, to act as a check upon the previous one, in order to confirm or contradict the surprisingly high figure for mutation rate there found, and, second, to procure early evidence, prior to any which might be forthcoming from the balanced lethal experiment, concerning the possible effect of temperature upon the rate of mutation. In the sex ratio work on the X-chromosome all the lines had been propagated at a rather warm room temperature (in Texas), and no attempt had been made to apply different conditions.

In the method used in the 1919 joint experiment, a reversion was made to the class work of the spring of 1918, which has already been described. It will be remembered that here the identifying factors, *w^e vf*, and their normal allelomorphs, were used, each culture involving a cross of the type *w^e vf* ♀ by *w^e vf* ♂. The presence of these identifiers permitted a lethal to be known by an easy "qualitative test"—the absence of all of a given class of sons, and no count was ordinarily required. This gave much more decisive results in the original "test-cultures" and far fewer temporarily doubtful cases, than did the sex-ratio method. It could in addition be determined at once in which chromosome of the female (the paternally or maternally derived) the lethal lay, and even what its approximate location in this chromosome was. Further, the daughters receiving their mother's lethal could be distinguished from the others by inspection, so that to be sure of perpetuating the lethal in the next generation it was not necessary to carry on the simultaneous breeding of any of their non-lethal sisters. This difficulty did arise in the generation following, however, in case the lethal lay in the *w^e vf* chromosome, for then the lethal-containing daughters ("F_1") of the original heterozygous female were of the homozygous recessive type, and so there was no way of telling which of *their* daughters ("F_2") had gotten the lethal; in this "F_2" generation, then, the supernumerary breeding of unknowns became necessary. Such a difficulty was never encountered, in any of the generations of testing, when the lethal was in the "wild-type" chromosome.

The chief disadvantage of this method, as compared with that involving sex-ratios, lay in the necessity of securing virgin females in order to be sure that the daughters which resembled their mothers phaenotypically were really like them genotypically, and hence suitable for further breeding. This entailed considerable extra labor, but not as much as was saved by the greater definiteness and speed of the lethal determination in the presence of "identifying characters."

The cultures in this experiment were kept in half-pint milk bottles. The "warmer series" were kept in an incubator at $27.0 \pm 1°C$. The flies in these cultures started hatching 8 days after the parents had been inserted, and the duration of the generations that was allowed here varied from 12 days to about two weeks, that is, the offspring were placed in fresh culture bottles after this length of time had elapsed since their parents had been placed in the old cultures. The temperature of the "cooler series" was differentiated from the other in sign, but not by a quantitatively constant difference, as the bottles were cooled by keeping them in shallow pans of running sea water (at Woods Hole). Thermometer readings showed that the temperature in these bottles averaged about 19.5°C. The offspring started hatching about 12 days after the parents had been placed in the culture bottle, and the generations were allowed to take from $2\frac{1}{2}$ to 3 weeks' time.

In the warmer series there were 517 females tested, in addition to the ancestral females of the preliminary tests for eliminating antecedent lethal mutations. Among these 517 females tested for new mutations, 13 lethals were found and no visible mutations. This rate of 1 lethal per generation in 40 females, or 1 in 80 X-chromosomes (1.26 percent), is obviously closely similar to the rate of 1 in 60 X-chromosomes found by ALTENBURG at the Texas room-temperature, and calculation shows that the observed difference between these two rates is only equal to 1.1 times its own probable error, a difference having, of course, no significance. In the cooler series, on the other hand, 445 females from non-lethal mothers were tested for new mutations, and among these only 5 lethals were found, and no visible mutations. This gives a rate of one lethal in nearly 180 X-chromosomes, per generation—0.56 percent—or less than half the rate in the warmer series.

It may be added that approximately 100 bottles, those in which the further tests of the lethals were being made, were kept at room temperature, and that, in these, two new lethals arose during the course of this testing. The X-chromosomes available for the detection of such new lethals numbered about 100 here, since one X-chromosome of each parent female (the "maternal" one) already carried a lethal.

The difference between the rates of mutation per X-chromosome, per generation, in the warmer and the cooler series ($1.26-0.56=0.7$ percent), was then compared with its own probable error as calculated by means of the formula previously given ($0.6745\sqrt{P/(n_1 \cdot n_2)}$). Here we have $P=18$, while n_1, the total count of X-chromosomes in one of the series, is 2×517, or 1034, and n_2, the other count, is 2×445, or 890. This reckon-

ing gave a probable error of 0.3 percent. Thus it turned out that the difference (0.7 percent) was 2.35 times its own probable error. If the rates of mutation per X-chromosome, per generation, under the two conditions actually employed, had really been alike, a difference of this magnitude, in this "expected" direction, would have occurred only once in 18 trials (and a difference of this magnitude in either direction once in 9 trials). This is obviously far from a convincing result (as has always been realized), yet it does give rise to a certain moderate presumption, or "probability," as termed in the original note, in favor of an effect having been produced, and the above numerical value of this probability cannot be reconciled with a criticism which has been made that the "data are clearly not statistically significant."

If now, instead of calculating the rate of mutation per X-chromosome, per generation, we reckon it per X-chromosome per day, month, or any other unit of time, we find a difference larger than the above between the rates in the two series (relative to their own values), since there were more generations in the warmer than in the cooler series, in a given time. On such a basis, we find 2.96 percent of mutation, per X-chromosome, per month, in the warmer series, and 1.06 percent in the cooler series, the difference here being 1.9 percent. The probable error of this difference proves to be 0.63 percent, so that this difference is 3 times its probable error. Such a difference would only occur, in the given direction (expected for most heat effects), in one trial out of 48, if the rates per month were really alike; such a difference, in either direction, would occur in one trial out of 24 if the rates per month were alike.

It is of interest to note that, in contrast to the difference in rate of mutation indicated between the warmer and the cooler series, no evidence of any significance was obtained for a difference in rate between male and female, for, of the 18 lethals which the females of the two series possessed, 7 were in the chromosome they had received from their mother, and 11 in the chromosome from their father. The 100 bottles at room temperature, which gave evidence only on the mutation rate in the "paternal" chromosome (since the other already contained a lethal), yielded, it may be recalled, 2 new "paternal" lethals, so that altogether there were 13 lethals in 1062 paternal chromosomes, or 1 in 82 (1.2 percent) to be compared with 1 in 137 maternal chromosomes (0.73 percent). A difference of this magnitude would occur somewhat oftener than once in 4 trials if the rates were really alike.

Before further experiments on the effect of temperature could be completed (and while the first balanced lethal experiment was being

carried on) the writer undertook to make an extended study of the possible identity and the positions of the loci involved in the mutations already obtained in this joint X-chromosome work. It was easy to map the loci approximately, by noting the relative amounts of numerical deficiency in the various crossover classes. The establishment of identity or non-identity of the loci of any two of the sex-linked lethals found to lie in the same region presented greater difficulty, since two such lethals cannot be crossed together (owing to the inviability of males with a sex-linked lethal). Direct evidence of identity of the locus of two lethal mutations in the X-chromosome can, in fact, be obtained only when both prove to be allelomorphic to the same non-lethal "visible" gene, to which both can be crossed separately.

Such a finding of mutual allelomorphism was obtained in the case of two of the independently arisen lethals. One of these was completely recessive to normal but threw "broad"-looking females after being crossed to "broad" winged males, and the other gave a somewhat "broad" wing and body in heterozygous condition (when with the normal allelomorph), and gave a lethal combination with "broad," the pupae becoming black and dying at an advanced age. These lethals, then, must both have been allelomorphs of "broad" and therefore of each other, but *different* allelomorphs, and the locus may be a relatively mutable one. This is especially probable because a visible mutation in the same locus, giving an extremely "broad" wing (more extreme than "broad" but not as extreme as the previously known allelomorph called "short") occurred in one of the secondary cultures in which the lethals from the main experiment were being tested out. Since these various allelomorphs are different from one another, the high mutability of this locus might be of a different kind from that in variegated corn or in DEMEREC's mutable races of *Drosophila virilis*, where, supposedly, "gene-elements" that have previously mutated are merely being sorted out. There is, moreover, no reason to believe that the apparently high mutability of this locus is connected in any way with the fact that the two mutant genes found here in the main experiment were lethals, since at least three different non-lethal mutations have also been observed to have occurred in the same locus (counting broad, short, and the new mutant above referred to). The finding, in such a locus, of two lethal mutations which, with the three visible mutations, form a series of decreasing viability and of simultaneously increasing somatic effects, indicates rather that the lethal mutations are essentially similar in nature to the others.

Another lethal proved to be an allelomorph of the previously known

visible gene for "facet" eye. Unlike the other lethal allelomorphs of facet, previously known, which have all been designated as "notch," it did not cause the "notch wing" effect. Four of the other lethals, though not allelomorphs of known visible genes, were themselves on certain occasions "visible," that is, a male bearing the gene managed to hatch in a small percentage of the cultures of the given type. One of these four genes, located between scute and prune, caused a tendency to "cloven" thorax in such males; another, between echinus and cut, gave an extremely "diminutive," sterile male; the third, between vermilion and miniature, caused very weak-looking males with characteristically "flimsy" wings and the fourth, between scute and prune, resulted in "collapsed" wings and in leg abnormalities.

The loci of all the lethals studied were (except for the two allelomorphs of broad) probably all different from one another; certainly most of them were, as was proved by tests that showed them to lie between different "visible" genes. The arrangement of the loci of these lethals, as approximately determined in relation to those of standard, visible mutants, is shown in figure 2. It will be noted that about half of them are concentrated

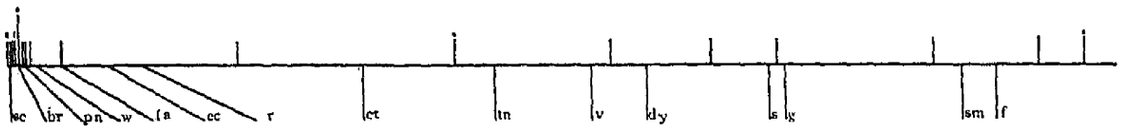


FIGURE 2.—Figure to illustrate mode of distribution of lethals arising in the X-chromosome, based on data from the first temperature experiment on this chromosome. Mode of representation as in figure 1, except that map is drawn to one and one-half times the scale there used.

in the very short region (1.5 units of the 70±) to the "left" of white. This agrees with the similar clustering that has been found in the case of the visible mutant genes in this chromosome, and may merely depend on a "foreshortening" of the map of this region, due to a lower frequency of crossing over here. It was in this region, therefore, that the determination of non-identity of the loci was less accurate in some cases.

It might be objected that all this work of mapping the lethals, determining their allelomorphism, and their possible somatic effects, was a digression from our main object of studying the mutation rate. It was important, however, that such studies be made in one experiment at least. For it was thereby demonstrated (1) that most of the mutations had occurred in different loci; (2) that these loci were grouped in a fashion similar to that known for "visible" mutant genes; (3) that the loci in some cases coincided with those of visible genes; (4) that the allelomorphs in such cases might form a graded series; (5) that there were some semi-lethals which connected lethals and visibles and indicated that there was

no absolute distinction between the latter in regard to the kind of effects produced. All this was desirable in order to show that in such experiments as these we are not merely dealing with a few anomalous genes, that are mutating repeatedly and in some peculiar way, but with chance-given samples of many genes, the mutations of which may probably be taken as representative of mutations in general, including "visible" mutations.

COMPARISON OF DATA OBTAINED ON MUTATION RATE IN THE FIRST AND SECOND CHROMOSOMES

The difference in rate of mutation, apparently associated with temperature, was in the same direction in both the experiments involving temperature differences which have thus far been reported. The results on the second chromosome, though presented first, were, it may be recalled, obtained later than those of the joint experiment on the X-chromosome, and so they could not be taken into account in the preliminary note previously referred to. We may here, however, consider them in combination with the results on the X. For this purpose a special mode of reckoning will be required; it will not suffice simply to make a total of the data in two experiments performed in a different way on different objects. Nor will any unweighted average of the results in the two experiments exhibit the full significance of the combined data.

One valid method of obtaining the most informative figure possible concerning the effect in the combined experiments is as follows: First, express the difference found between the two series in terms of its own probable error as a unit, in the case of each experiment separately. This puts the results of the two experiments into comparable terms. For the X-chromosome, it will be recalled that the difference in mutation rates per chromosome-generation was 2.35 times its probable error, and for the second chromosome 1.6 times its probable error. Now, the chance of obtaining a combination of results at least as improbable as this combination is the same as that of obtaining, in a single experiment, a result equal to the square root of the sum of the squares of these individual values. Making the latter calculation (by taking $\sqrt{(2.35)^2+(1.6)^2}$) we get the figure 2.84. The chance of obtaining, in an individual experiment involving random sampling, a result this many times its probable error is 1 in 17+, or if we consider the direction of the difference as specified, 1 in 35. In other words, there would have been only 1 chance in 35 of getting a combination of differences as "improbable" as that observed, and in the direction of an increase accompanying heat, if the temperature difference, *or some agent accompanying it*, had not really affected the mutation

rate per chromosome, per generation, in *at least one* of these two experiments. Such a result would ordinarily be regarded as furnishing moderately strong evidence for an effect.

The same method of reckoning may be applied to the figures for the mutation rate as measured in terms of "chromosome-months." In the first chromosome the difference was 3 times its probable error, and in the second chromosome almost the same—2.9 times. Calculating as before ($\sqrt{3^2+(2.9)^2}$) we find that the probability of such a combination of differences is the same as that of a single difference 4.15 times its own probable error. An event as improbable as this would occur in only one trial out of 197, regardless of the direction of the difference, or in one trial in 394, in the given direction. It may therefore be considered as "proved" by this work that, in at least one of these two experiments, there was a difference in the time-rate of mutation between the warmer and the cooler series brought about by some cause other than the fluctuations inherent in simple random sampling. It was difficult to believe that the cause could be other than temperature, acting either in a "direct" or in some indirect fashion, since the cultures in the warmer and cooler series had been sensibly alike in other environic respects and it was scarcely to be expected that such chance invisible gene differences as might have been possible here would influence the general mutation rate throughout a chromosome.

There was, however, one conspicuous feature of the work which gave rise to doubt and conjecture on the latter questions. The mutation rate for the X-chromosome was markedly higher than that for the second chromosome, both in the warmer series, which were kept at almost the same temperature in the two experiments, and in the cooler series. The difference should, seemingly, have been in the opposite direction, since the X is only two-thirds as large as the second chromosome (both cytologically and also as measured by the length of the linkage maps). Allowing for this difference in chromosome size, we find that, in the two warmer series alone, the difference between the mutation rates of the two chromosomes, per unit length—reckoned as 13 in (1034×70), and 24 in (4098×105), respectively—is 5.4 times its own probable error, no matter whether "chromosome-unit-generations" or "chromosome-unit-months" are considered. We must therefore conclude either that the mutation rates in the two chromosomes differ, even when they are subjected to identical conditions—this would be most curious—or else that there were differences in other conditions than temperature (either environic or genetic) that caused the difference in the mutation rates of the two experiments. This latter possibility, though it seems the less remote of the two,

would appear strange enough, and yet one or the other of these two conceptions must be correct.

If, now, it was true that such differences in cultural conditions or in genetic composition as distinguished these two experiments could result in the observed difference in mutation rate between them, might it not be true also that some similar environic or hereditary difference happened to exist, unbeknown to the experimenter, between the cultures of the warmer and the cooler series, in one or both of the experiments? In that case, this condition might have been responsible for the significant difference observed between the two series, and we should be wrong in attributing the effect to temperature. How, then, could the possibility of such an influence be avoided? One obvious way was by a repetition of such "temperature experiments," since of course if a result in the same direction was consistently obtained in enough experiments, the influence of factors other than that which had been consistently varied throughout all the experiments alike would finally be excluded. Meanwhile, however, even before the results of the second chromosome experiment had been obtained, other experiments, on the X-chromosome, had in fact been initiated, in the hope of getting further evidence. These experiments, and those that followed them on the X-chromosome, will now be described, as they had an important bearing on the point here at issue, and showed (by a process of elimination) what features the definitive temperature experiment would have to possess.

THE DISCOVERY OF SIGNIFICANT VARIATION IN THE MUTATION RATE, OF UNKNOWN ORIGIN

During the academic year 1919–20, while the lethals found in the joint experiment of 1919 were being mapped, and the cultures involving the second chromosome were being carried along (but not yet tested), crosses were also being made in preparation for an experiment whereby it was hoped that a much larger, more decisive mass of data might be secured, concerning mutation frequency in the X-chromosome at different temperatures. To this end, the writer synthesized an elaborate X-chromosome stock, which was intended to make the determinations and tests of the lethals still easier and more definite, and especially to facilitate the propagation of the lines from generation to generation, by making it unnecessary to secure virgin females. Non-disjunction, too, was allowed for, so that its occurrence would neither interfere with the lethal determination nor make it necessary to secure virgins. In cultures derived from

these lines, moreover, the "supernumerary breeding of unknowns" would in no event be required.

It would be superfluous to detail the genetic circumstances which, theoretically, were to bring about these results, but the composition of the stock synthesized may be recorded here, as follows:

(odd generations) $\frac{y\ w^e\ c_v\ c_t\ v\ g\ f}{s_c\ w^e \qquad t_n\ \ s_m} \varphi \times s_c\ t_n\ v\ s_m\ B\ \sigma$

(even generations) $\frac{y\ w^e\ c_v\ c_t \qquad v\ g\ f}{s_c \qquad\quad t_n\ \ v\ s_m\ B} \varphi \times s_c\ w_e\ t_n\ s_m\ \sigma$

Some thousands of cultures of these types were bred, in joint work of ALTENBURG and the writer, in the summer of 1920, under purposely varied temperature conditions, but the experiment failed because, under the conditions of rearing at Woods Hole, the males containing the genes of the $yw^ec_vc_tvgf$ chromosome, and to a lesser extent those of the other types, had such low viability, even when no definite lethals were present, that the number of "doubtful" cases arising became too large to deal with. The work had to be stopped before nearly all these cases could be resolved—a situation which of course destroyed the significance of the entire experiment.

But from the ruins of this experiment there issued suddenly the hope of a much more effective attack upon the problem. For in one of the last cultures examined by the writer a mutant condition called "C_l" was found, in the paternally derived $s_ct_nvs_mB$ chromosome of a female, which was at the same time (recessive) lethal in its effect, and prevented nearly all crossing over between this chromosome and its not similarly mutated homologue. This "mutation" appeared to me to offer unexampled technical advantages for further mutation work on the X-chromosome. The reasons for this may be explained in detail, as considerable use has been made of the method involving this mutant chromosome, both in the work herein to be reported and in other mutation studies, to be reported elsewhere.

In the first place, the suppression of crossing over caused by the mutant chromosome is highly advantageous. For the occurrence of crossovers seriously hampers the determination of whether or not a lethal is present (even though crossovers are eventually necessary for determining its locus), since only those character-combinations will be uniformly absent from a count which depend on visible genes that lie in their original arrangement on either side of the lethal, that is, it is for the absence of

given non-crossover types that one must look, primarily. We must make an exception here in the case of such specific crossing over as may be necessary in experiments like the one on the second chromosome, previously described, where a certain "antecedent lethal" has to be removed by crossing over before new lethals can be recognized. Even here, however, the new lethal can be recognized only by the absence of flies homozygous for chromosomes that are non-crossovers in the region of this new lethal. The occurrence of most crossovers, then, reduces the number of offspring available for the lethal determination, making the latter less decisive. In addition, the presence of these crossovers makes necessary a much more detailed inspection of the flies, in order that the presence or absence of the crucial non-crossovers may be ascertained.

Secondly, the presence of a lethal in the same X-chromosome of the female as contains the "factor" preventing crossing over, although it leaves only the other X of the female available for the study of new lethals, causes all counts from females containing a new lethal in this other X to exhibit a 1 ♀ :0 ♂ ratio. For the antecedent lethal in the first X-chromosome will kill half the sons, while the new lethal in the homologous X will kill the rest. On the other hand, females in which a lethal has not arisen in this homologous X will throw a 2 ♀ :1 ♂ ratio. We have, therefore, in cultures of such stock, only to distinguish between a 2:1 and a 1:0 sex ratio rather than between a 1:1 and a 2:1 sex ratio, as in the ordinary case, and the former distinction (involving a kind of "all-or-none reaction") is of course much surer, more definite, and more readily determined, than the latter. All that is necessary, then, in testing for a new lethal in a female carrying C_l, is to see whether any males at all are present among the offspring. One does not, ordinarily, even have to distinguish the "identifying characters" of the males. This makes it possible for the determination of the lethal, in most cases, to be made merely by inspection with the naked eye, or with a hand lens, through the glass wall of the culture vessel, without etherization of the flies being required. It is true that an occasional male will appear, even in lethal cultures, made possible by primary non-disjunction or by the sporadic crossing over that takes place, but such lone males can then be further examined, and the cross can have been made in such a way that they will readily reveal their origin by conspicuous "identifying characters," visible through the glass container.

A third advantage of having one of the mother's X-chromosomes contain a known antecedent lethal, and unable to cross over with the other X, is that this results in only one kind of male offspring being produced

(barring the above-mentioned rare crossovers and non-disjunctional exceptions); as the compositions of the parents can be so arranged that this type of male will be suitable for the daughters to mate with, for the continuation of the experiment, it will then be unnecessary to obtain virgin flies, a procedure that otherwise occupies perhaps a third of the working time.

Fourthly, in the C_l-containing chromosome in question, the "visible" mutant genes present, s_c, t_n, v, s_m and B, afforded excellent "identifying factors," so that it was feasible to use, in the homologous chromosome in which new lethals were to be looked for, a contrasting gene-combination possessing relatively high viability. In this way the number of "doubtful cases" might be reduced to a minimum. As a matter of fact, it was decided to use a $w^{co}fB$ (coral forked bar) X-chromosome in this place in some cultures, and in the others one containing the combination $w^{e}vf$; the high viability of these had been proved in preliminary experiments. A scheme was arranged whereby, in each line, the $w^{co}fB$ and the $w^{e}vf$ chromosome exchanged places in alternate generations, as this procedure made it possible easily to recognize any flies resulting from non-disjunction. This scheme of breeding was as follows:

odd generations $\dfrac{s_c\ t_n\ v\ s_m\ B\ C_l}{w^e\quad v\quad f}$ ♀ $\times w^{co}\ f\ B$ ♂

even generations $\dfrac{s_c\ t_n\ v\ s_m\ B\ C_l}{w^{co}\quad f\quad B}$ ♀ $\times w^e\ v\ f$ ♂

It will be seen that such stock perpetuated its alternating composition, and was always ready for lethal tests, without virginity being specially sought for, provided only (1) that the parents were discarded before the offspring hatched, (2) that non-disjunctional or crossover males did not appear, and (3) that a single heterozygously or homozygously bar-eyed female (according to the generation) was taken, with any of her brothers, to start each culture. No "preliminary tests" were necessary since in the paternal chromosome—which alone was studied—there could originally have been no lethal (or the father would not have existed). If in any culture the existence of a new lethal was discovered, by the absence of males, the daughters having the lethal could be distinguished from the others. They would have the composition $\dfrac{w^e\ v\ f}{w^{co}\ f\ B}$. These females could then be crossed to any males desired, for further testing of the lethal, as they were necessarily virgin (having no living brothers). As they con-

tained "identifying genes" scattered through their X-chromosomes the cross could be made so that their daughters receiving the lethal could also be recognized, and there need not be any "supernumerary breeding of unknowns" in any generation. But since these $\frac{w^e\ v\ f}{w^{co}\ f\ B}$ females did not themselves contain the "factor" that prevented crossing over, the families derived from them gave immediate data on the locations of the lethals.

A final highly important advantage lay in the fact that these cultures could be reared in 4 by 1 inch vials, rather than in milk bottles, a feature which allowed the preparation and handling of many more cultures. The use of vials, with their smaller total counts, was rendered possible by the fact that, owing to the absence of crossovers, the proportion of flies which gave evidence regarding lethals was greatly raised, as has been explained, and by the further fact that chromosomes could be employed that allowed the males (if non-lethal) to have a high viability.

All these favorable features very much more than compensated for the fact that twice as many cultures were now needed in order to obtain a "mutation count" of size equal to that gotten previously (owing to the limitation that only one chromosome of the female—the "paternal" one—could be studied). It would have been of interest to compare the mutation rates in both maternal and paternal chromosomes, as before, and the limitation of the count to the paternal chromosome was in this respect a drawback. However, there seemed no reason why the fact that the chromosomes tested had all been contained in the male in the generation preceding the test should work seriously against the obtaining of a significant total count, because the earlier experiment had given at least as high a mutation rate in the paternal as in the maternal chromosome.

In accordance, then, with these favorable indications, the writer synthesized the alternating C_l stock above described, multiplied it many fold, and then carried out tests upon it, at the UNIVERSITY OF TEXAS, in the winter and spring of 1921, during twelve fly generations. Throughout this time all the cultures were kept in the incubator at a temperature of 27°C, as it was desired to obtain definitive data for the "higher" temperature first. In the entire experiment at this temperature, a total of 3935 cultures was examined, representing the same number of tested "paternal" chromosomes. The cultures were of two kinds, inasmuch as in 3438 of them the previous generation had been allowed to occupy the customary 10 to 14 days, but in the 497 other cases the female chosen for testing was one in the mother of which the sperm had been "aged" for

a week or more before the fertilization occurred which produced the fly that was tested. The object of the latter special procedure was to obtain evidence as to whether during such aging mutations would occur.

As culture after culture of this experiment was examined, sons of "regular" type continued to be noted in almost all of them until, after the above number of nearly four thousand cultures had been tallied off, it was found that only four lethals, in all, had appeared among them! None of these lethals happened to be from the "aged" sperm.

The above surprisingly low number of mutations was not caused by any lethals having escaped detection, for there were exceptionally few doubtful cases and all of these were eventually resolved. On the contrary, the above number may in one sense be considered as too high, since 2 of the 4 lethals were almost certainly of identical origin; they occurred in sister flies and had, so far as could be ascertained, the same locus. This appearance of two lethals having a common origin was made possible by the fact that the experiment involved a departure from the principle of breeding only one daughter from each parent culture: instead, an average of 8 daughters were bred from a parent culture, and, in compensation, only 1/8 of these filial cultures were then continued further, in the generation succeeding them; this same procedure was followed out in each generation. It is permissible to do this where pedigrees "within the lines" can be kept, and where the tests are made and recorded in each generation (which cannot be done where the lethals are accumulated in balanced stocks). It involves us, however, in certain difficulties in the computation of the mutation rate, as a somewhat larger probable error is then applicable, due to the resulting correlation between the lethals appearing among sisters. Still, the error is not very much larger than in random sampling, because mutations that appear singly even then usually remain in large majority (BRIDGES 1919, and MULLER 1920).

Taking the figure 4 as representing the number of newly arisen lethals, we find a mutation rate of only 1 lethal in a thousand "paternal" X-chromosomes (including, it will be remembered, in an eighth of these cases, chromosomes derived from sperm that had been aged for nearly the length of a zygote-generation). The difference between this rate of 0.1 percent and the rate of 1.2 percent, obtained from the finding of 13 lethals among 1062 paternal X-chromosomes in the earlier joint experiment (including here even the cooler series), is 8.2 times its own probable error, if we calculate the latter by the usual random sampling formula (namely, $0.6745\sqrt{P/(n_1 \cdot n_2)}$). Even if the error should really be taken as twice as large, then, this is an absolutely decisive difference—despite the com-

paratively small number of mutations involved in each case. And it was obtained, be it again noted, in the face of the higher average temperature at which the later experiment was conducted.

Aside from such differences as might have been caused by the use of vials in place of bottles the cultural conditions in the two experiments in question were closely similar. They were almost certainly more alike, in general, than the conditions in ALTENBURG'S sex-ratio experiment in Texas and those in the earlier joint experiment at Woods Hole—the results of which had nevertheless agreed closely. On the other hand, it was also difficult to conceive of the cause of the difference in mutation rates as having been genetic. For, in the work with C_l, the two classes of fathers used in alternate generations had had a very different origin, and one of them—*w*^{e}*vf*—was derived from the very stock that had furnished the recessive chromosome in the earlier temperature experiment. As for the dominant chromosome in that experiment, it had, in part of the cultures, been derived from a very different source from that concerned in the other part, without a difference in mutation rate occurring between the two parts. Thus there seemed to be no consistent genetic difference between the cultures in the C_l experiment and those in the earlier joint experiment, to explain the consistent difference in mutation rate. It should further be noted that the C_l-containing chromosome itself had, prior to the mutation by which C_l originated, been used in the later joint temperature experiment, and that in this also, although too many doubtful cases had arisen for an exact mutation count, there had nevertheless clearly been no dearth of real lethals. The C_l-containing chromosome itself could scarcely have exerted any influence on the mutation rate anyway, because only mutations in "paternal" chromosomes, derived from males not containing C_l, were studied, and these mutations must in most cases have occurred in those males.

So small had been the number of mutations in the experiment with C_l that it was obviously impracticable to pursue the original plan of following this with an otherwise identical experiment in which the cultures were kept at a lower temperature. For no significant difference due to temperature could reasonably be expected in such small numbers. Since, moreover, some unknown environic or genetic difference had been able to cause a significant difference between the mutation rates in the experiments already performed, it might again enter, in case of a repetition of the C_l experiment, to produce a conspicuous effect that might incorrectly be attributed to temperature. Thus the attempt to obtain evidence concerning the effect of temperature had again been obstructed. And it appeared

more important, now, to investigate further, or at least to obtain further corroboration of, those large variations in mutation rate, of unknown origin, that had just been met with.

CORROBORATION OF THE UNEXPLAINED DETERMINATE VARIATION

With the above end in view, it was decided to conduct an experiment which should be largely a repetition, on a greater scale, of the earlier joint temperature experiment, but this time without temperature differences, simply in order to determine whether or not figures for the mutation rate substantially like those gotten in that experiment would again be forthcoming. This new experiment was carried out at the UNIVERSITY OF TEXAS, in the fall and winter of 1921–22. It was in part financed by a grant given by the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE in 1921.

As in the experiment of 1919, the initial crosses ("P_1" generation) consisted of matings of homozygous recessive eosin vermilion forked ($w^e vf$) females, in pairs, to dominant (in the main, wild-type) males. In the three following generations, in which the definitive lethal tests were made, heterozygous females $\frac{(w^e vf}{\quad}$ or sometimes $\frac{w^e vf}{B}$, as will be explained) were crossed, in pairs, to their triply recessive ($w^e vf$) brothers. There were 28 of the original (P_1) cultures. In those numbered 1 to 26 the eosin vermilion forked females were derived from the "regular" stock having this combination of genes, but in those numbered 27 and 28 they were derived from "$w^e vf C_{1A}$" stock (a stock in which BRIDGES had found secondary non-disjunction to be increased and crossing over diminished). In cultures number 1 to 13, inclusive, the dominant males were of normal stock from Florida. In numbers 14 to 26, inclusive, they were of normal stock from Falmouth. In cultures 27 and 28 the dominant males were bar eyed, otherwise normal-appearing, and were homozygous for "C_{1A}," being derived from the same stock (maintained by perpetual selection and crossing) as their $w^e vf$ mates.

The number of P_2 cultures derived from each of the 28 P_1 cultures is shown in table 1. In all, 678 fertile P_2 matings of the heterozygous females were started. As these females occurred in groups of sisters, there was a chance here for lethals of common origin to occur. In the next generation (P_3), however, not more than one pair was taken from each of the above (P_2) cultures, and in P_4 likewise this system was followed. There were 604 fertile P_3 cultures started and 437 fertile P_4 cultures. The reason for the

drop in numbers in this last generation was chiefly because here pairs were only taken from those P_3 cultures in which the mothers (P_3) proved to have been virgin before the mating of the desired type had been made; otherwise P_4 females of the wrong genetic composition might have been obtained. (In the preceding generations, as can easily be worked out, non-virginity would not have interfered with the testing.) The P_1 cultures were raised in bottles, and all the later cultures in vials. They were kept in the incubator at 26.5 ± 0.5°C, and 14 ± 1 days were allowed to a generation.

None of the 28 P_1 females proved to have contained a lethal, as the sex ratios showed. Among the P_2 females tested there were 4 lethals. Of these, two may have had a common origin as they occurred in the same (paternal) chromosome of sisters, and both were situated very near *W* (the normal allelomorph of w^e), in loci that may have been identical. The others in this generation were of separate origin, and those of subsequent generations must have been so, owing to the breeding methods used. Among the P_3 cultures there were 4 lethals, and among the P_4 cultures 2. In all, 6 of the 10 lethals occurred in paternally derived and 4 in maternally derived chromosomes. Seven of them arose in the series derived from the cross of Florida males, 2 in the series from the Falmouth males, and 1 in the "C_{IA}" series. The details concerning them are given in table 2.

If we include in our reckoning only the P_3 and P_4 cultures, since only in these had the lines of descent been kept separate during the preceding generation, we find 6 lethals in 1041 females, that is, in 2082 X-chromosomes, or 1 lethal in 347 X-chromosomes. This gives 0.3 percent of mutation. The inclusion of the P_2 generation in addition would give the figure of 10 lethals in 1719 females, or in 3438 X-chromosomes. This yields the almost identical result of 1 lethal in 344 X-chromosomes, or, again, 0.3 percent of mutation. It will be observed that this rate is markedly below that obtained in the earlier joint experiment, but noticeably above that in the work with C_l.

If, now, we treat the data as a collection of completely independent samples, using the "probable error of a difference" formula based on "simple sampling" ($0.6745\sqrt{P(n_1 \cdot n_2)}$), we find that the difference, 0.65 percent, between the present result of 0.3 percent and the result (0.95 percent) obtained in the earlier joint experiment as a whole (warmer plus cooler series) is 5.7 times its own probable error (or 3.9 times if only P_3 and P_4 are included), and is hence "significant." The difference between the present result and that of 0.1 percent, found in the experiment in-

volving $C_{,,}$ is 2.8 times itsown probable error, if the latter is obtained by the method just referred to; accordingly this is "probably significant" also. If, on the other hand, the data cannot legitimately be treated as a collection of independent samples, this in itself implies determinate differences in mutation rate between different groups of the cultures within the experiment, and so we reach the same conclusion: that is, that significant variation in mutation rate is occurring, due either to environic or genetic causes.

Examining now the distribution of the lethals we notice at once that four of the ten occurred in the descendants of one of the 28 original P_1 pairs (number 1), although only one-twentieth of the tested females were derived from this pair. Such a concentration of the lethals within one group scems well beyond the limits of a purely random distribution. None of the four lethals could have originated by the same mutation, although two of them, which were in the recessive chromosome very close to w^e, might be conceived to have been a later result of some "premutation" (see footnote, p. 293) that occurred in the common ancestral chromosome. Even if we rule out one of these on account of this possibility the concentration of separate lethals here is still beyond what would be expected in a random distribution. In the group from P_1 pair number 7 we find a situation that appears similar: three lethals in a total of 141 tested females. Here, however, the first two of the lethals, both occurring in the paternal chromosome of P_2 females, in possibly identical loci, may well have been of common origin. If we count them as one, there still would be two lethals in this group, a rather unlikely occurrence for random sampling in an experiment where lethals in general are so rare. The third generation lethal in this group also might be conjectured to have been related to the others by "premutation," as all of them lay in the dominant chromosome, at loci that may have been the same; hence, although "premutation" is of very questionable occurrence in Drosophila, we cannot use the results from group 7 to prove a tendency to an increased general mutation rate, as we can those from group 1. Nevertheless, these two groups (both from the cross of Florida males) certainly corroborate each other, in indicating that some groups mutate significantly more often than others which have and have had apparently the same cultural conditions and "visible" gene composition.

Selecting just these two groups, we find in them, taken together, 7 lethals in 456 X-chromosomes, or 1 in 65 (1.5 percent), which is a rate that agrees closely with that observed in ALTENBURG'S sex-ratio work and in the earlier joint experiment at the higher temperature. On the

other hand, all the other groups, taken together, showed only 3 lethals (all different) in 2982 X-chromosomes, a rate of approximately 1 in 1000, or 0.1 percent, like that found in the work with C_l. And the present experiment, taken as a whole, showed, as has been remarked, a rate significantly different from that found in either of these two previous experiments. Thus, whatever the cause of the variation in mutation rate may have been, the present experiment confirms the conclusion regarding its actuality.

There were certain important lessons to be drawn from these empirical facts, even though their cause was yet problematical, and while it was even doubtful whether genetic or environic factors were responsible, First, it was obviously inadequate to follow the original plan of obtaining data from "controls," all supposedly under one set of conditions, in one year, and data from a group of "treated flies," reared under a purposely different set of conditions, another year. For elusive genetic differences between the two groups, not reflected in any difference in their known formulae, might have cropped up by mutation, or have been spread through differential breeding, in the course of several months, and these might, for all that was yet known, become the cause of any observable changes in the mutation rate. On the other hand, if the previously found unexplained variations in mutation rate had not been due to such genetic causes, then, whatever the environic influences may have been that had unsuspectedly been responsible for the effects, these too might again arise to differentiate two series of cultures run at different times in our projected later experiments. To be sure of avoiding both possible complications, then, the two series would have to be carried along simultaneously, even though this might entail extra labor. They would have to be so *related*, genetically, that *no possible hereditary differences* between them, known or unknown, could account for a difference found in their mutation rates and so *treated* environically that, aside from the agent in question, *no possible external differences* could account for the result.

Second, the C_l-containing stock, despite the advantages it presented for testing and for propagation of the cultures, could probably not be used to advantage for studying such differences in mutation rate as might be caused by different temperatures, as its ordinary mutation rate was, and might again be, too low. Similar conditions might, however, be found at any time (so far as the experimenter could predict) in any other stock. Hence it seemed desirable to use methods that would, with the same labor, allow still more wholesale testing for mutations than any methods previously devised for the X-chromosome.

After these trials of the possibilities of the X-chromosome, it therefore appeared as though the investigation of such (probably) moderate effects as those of temperature might be prosecuted more effectively by a resumption of intensive work with the second chromosome, where the accumulation method could be used. As we have seen, suggestive results had meanwhile been obtained from the experiment first started on this chromosome. The balanced lethal method, though requiring a considerable lapse of time between the initiation and the winding up of an experiment, had allowed the experimenter, with the aid of an assistant (more of the labor being routine in this method) to raise even larger numbers than had the "C_l" method. In addition, the amount of chromatin, and the probable number of genes involved in each chromosome tested, was nearly half again as great, since the second chromosome is $1\frac{1}{2}$ times as large as the X. It is true that, in spite of these advantages, the number of mutations found had not been great enough to give the results already obtained the full significance desired. Nevertheless—and these were the most important considerations—the method was found to be capable of being modified so as to become far more productive than it had been before, for a given amount of skilled labor, and, at the same time, it was found to lend itself readily to the securing of adequate controls, in the sense previously explained.

FURTHER MODIFICATIONS AND TESTS OF THE METHOD OF BALANCED LETHALS

ALLOWING FOR GENETIC AND CULTURAL HETEROGENEITY

It might at first sight appear a difficult matter to elude invisible gene differences and secure, for the different series of an experiment, material that would be sufficiently comparable genetically. Even stock that is originally quite homogeneous is, of course, subject to differentiation through mutations, invisible as well as visible. However, it is possible readily to avoid the effects of genetic diversity simply by following the plan of picking the ancestral individuals, for the lines of the different series, at random from the same original lot. For in that case the possible genetic differences between the individuals, that might influence their mutation rate, would be distributed between the two series according to the laws of random sampling, and could not affect the series differentially to an appreciably greater extent than would be allowed for anyhow, when the ordinary probable error formula was applied to the mutations. This effect depended on the fact that in each series there were a great number of lines,

and that only in a negligible proportion of cases did more than one separable mutation occur in a single line.

The differential effect of possible genetic differences on the two series could be still further reduced, moreover, by establishing the lines before the series were separated, and then forming the two series by splitting each original line into two or more divisions, of which half were placed in one series, and the other half—chosen at random from among the divisions of each original line—in the other series. This was equivalent to "grouping" the lines, a practice already in use to avoid preliminary testing, as previously explained, and to then dividing each group equally among the two series. As carried out in the present experiments, the attempt was made to have at least 100 such groups, and to have at least 4 "sister lines" in each group at the start, in order to make sure that two at least survived to serve as tests of each other. In the case of each group of 4 or more lines, then, 2 (or more) were placed in the "warm" series, and the other 2 (or more) in the "cool" series.

In regard to the problem of overcoming the effects of cultural heterogeneity similar considerations applied. If the two (or more) series of the experiment, which were run for comparison with each other, were carried on at the same time and in practically the same place (where vibration, radiation, barometric pressure, etc., were alike, except in so far as temperature itself might affect these), then all the differences in cultural conditions surrounding the flies in the different containers would be classifiable under the following two heads: (1) the differences in temperature itself and in all conditions (for example, state of the food) that are influenced by temperature under the circumstances in which the cultures are ordinarily maintained, (2) "chance" differences—for example, in regard to amount of food, tightness of stopper, etc.—which are independent of temperature and which a culture in one series is as apt to be affected by in a given direction as a culture in the other series. By the very nature of the conditions under the second head, these will tend to become "evened up" amongst the hundreds (or rather, in this case, amongst the thousands) of individual cultures of the two series, like the genetic differences previously postulated, in such a way that our random-sampling formula, when applied to the mutations finally found, will automatically allow for the practically random effects of these agents. This will leave only the agents mentioned under the first head, to account for "significant" differences in the mutation rate, that is, we can then be sure that any "significant" effect has been caused, either directly or indirectly, by temperature itself. Whether "directly," or "indirectly"—through the other conditions that

are somehow themselves affected by temperature—is of course another question, and one that the experiment by itself cannot pretend to answer.

Be it noted that in thus tracing the cause of an observed significant difference down to temperature or its necessarily associated conditions we have avoided the pitfall that lies open in the interpretation of other experiments, in which the cultures either are not carried on at the same time, or are carried on in places the environic conditions of which have not been carefully guarded. For, in either of the latter contingencies, there might have been differences, such as in light, radiation, quality of food, etc., that consistently distinguished the two series, even though these differences were not *causally*, but only *incidentally*, associated with temperature. It is the possible effect of such agents that we may avoid by means of our precautionary measures.

FACILITATING THE FINAL TESTING OF LETHALS

It was found possible considerably to reduce the labor in the final testing, and so to increase the numbers to be tested, by making use of the chromosome containing "curly," which had just been discovered and analyzed by WARD. For curly wings, which is a definitely dominant "visible" mutant, and was then associated with a recessive lethal, lay in a chromosome containing much more effective "*C* factors" (inhibitors of crossing over) than $C_{II\ L}$ and $C_{II\ R}$. The latter, it will be remembered, allow an appreciable amount of crossing over in the central portion of the chromosome, which lies between their respective regions of influence. For this reason it was necessary, when they were used, to have a lethal or sterilizing gene in each half of the test-chromosome. As no adequate sterilizing gene was available for the left half, star—a lethal—was used there, and when the final tests were made this had to be removed by a rather rare crossing over in order that the presence of new lethals might be detected. But by substituting the lethal, crossover-inhibiting, "curly" complex, for the $C_{II\ L}C_{II\ R}$ chromosome of the balanced stock, it was possible to make the balancing chromosome, and its homologue, each hold together as a unit, so that only one lethal or sterilizing gene was necessary in the chromosome to be tested. It was chosen to use the sterilizing gene, morula, for this purpose, and to eliminate the lethal, star, from the formula, in order that later, when the final tests were made, it would not be necessary to outcross in such a way as to allow crossing over. Then, if no lethal were present, the entire non-crossover combination, $T^d bp_r ca_r m_r$, would be able to manifest itself in homozygous condition, in one quarter of the "F_{n+2}" flies (barring differential viability), whereas

if a lethal were present there would be no such flies at all. Thus the number of flies per culture which gave testimony to the non-existence of a lethal was vastly raised, and, under fair viability conditions, the number of cultures at first recorded as doubtful could be reduced greatly below the number encountered in the preceding balanced lethal experiment.

The new balanced stock accordingly had as the basis of its composition for this work the formula:

$$\frac{T^d \;\; b \qquad p_r \;\; c \;\; a_r \;\; m_r}{C_y \, l_{CyL} \, C_{CyL} \;\; c_n{}^2 \qquad C_{CyR}}$$

Here C_y represents the gene for curly, C_{CyL} and C_{CyR} are the "C factors" preventing crossing over in the left and right half of the chromosome, respectively, l_{CyL} is the associated recessive lethal, and $c_n{}^2$ is an associated recessive gene for "cinnabar-2" eye color. Flies of this composition appear normal, except for their curly wings, and are very vigorous and fertile. In some cases cinnabar (c_n), an allelomorph of $c_n{}^2$, was substituted for purple eye (p_r) in the upper chromosome; the flies then had the bright red "cinnabar" eye color.

To make the final tests of balanced lines it may be recalled that a single fly (preferably a male) is taken from each line in the "F_n" generation and outcrossed in some way; F_{n+1} flies containing his test-chromosome are then bred *inter se*, and the F_{n+2} are examined for the presence of the multiply recessive flies. Various possible crosses are feasible in the first generation of this test, but the one which experience has proved to be most suitable is of the given male to a female having the following composition, specially synthesized for the purpose:

$$\frac{S \;\; T^v \;\; b \qquad p_r \;\; c \;\; a_r \;\; m_r}{C_y \, l_{CyL} \, C_{CyL} \, p_r \, c_n{}^2 \qquad C_{CyR}}$$

In the "F_{n+1}" from this cross the flies containing the test-chromosome from one parent and the curly complex from the other parent are readily distinguished from the rest by their having the combination: curly wings, non-star eyes, and purple or cinnabar eye color (according to which of these genes lies in their test-chromosome); the other curly-winged flies are star, and at the same time red eyed. In addition, flies will be formed that receive both chromosomes containing the similar "visible" recessive genes, and these will show all six of the corresponding recessive characters. They differ from the homozygotes to be looked for in F_{n+2} by also having star eyes, and by having long (non-dumpy) wings (although they show the vortices associated with dumpy). The apparition of these recessives

in F_{n+1} is valuable as a verification of the composition of the male from the experimental line, for if he is the result of some previous contamination of the line, these combinations will not appear, and the line must be discarded; thus one kind of later "doubtful case" will be avoided.

In choosing the F_{n+1} flies for mating, it is not absolutely imperative to secure virgins (though the youngest looking females should of course be selected), because, in the absence of crossing over, there is no chance for the test-chromosome to become "contaminated," and flies carrying it and curly can again be recognized and bred in the next and in each following generation in case lack of virginity (which will be apparent in the character of the offspring) should have been the cause of the non-appearance of the homozygotes sought. After virgins of the specified type have thus finally been obtained, in some generation or other, the stock derived from them will not only afford a test for lethals, but it will itself constitute a "self-perpetuating" balanced stock in which the lethal can be held indefinitely, without selection being required; this stock can then be used, at the experimenter's convenience, for any further testing of the lethals—including the test of direct crossing with other lethal stocks to determine possible allelomorphism of the different lethals. Thus the elaborate procedure, involving crossing over, etc., that was previously necessary for obtaining a readily useful stock of the lethal, is avoided. For determining the locations of the lethals, however, simple out-crosses of such stocks to stock containing a chromosome without "*C* factors," followed by inbreeding of the non-curly offspring, are necessary.

It will be seen that by means of the above method, although etherization and selection of the F_{n+1} are still necessary, the procedure is, all in all, very considerably simplified. In fact, if the determination of possible "visible" mutants is not an object, most of the examinations of F_{n+2} bottles for lethals can be made without etherization, by inspection of the flies through the glass of the culture bottle. For the six recessive characters will appear in almost inseparable combination, and thus the homozygotes are very easily distinguishable by the naked eye from their curly winged, otherwise normal-appearing sibs. Recessives resulting from non-virginity, and so carrying T^v in place of one of the T^d genes, are also plainly distinguishable, by their much longer wings, from the typical homozygous combination sought.

SIMPLIFYING THE ESTABLISHMENT OF THE LINES

Some time after this improvement was made in the final testing it was found possible to make an even more radical simplification in the pro-

cedure whereby the lines are started. This simplification was gained by an increase of genetic complexity, which, once established, was self-perpetuating.

The chief object in making the crosses and selections whereby the groups of lines were established was to insure the "unity of the source" from which all the test-chromosomes in a given line or group of lines were derived. Hence, a single male, heterozygous for the test-chromosome, had to be mated, and the offspring which showed, by their "identifying characters," that they had received this chromosome together with the required "balancing" chromosome from the female, had to be selected and bred *inter se*—virgins being eventually necessary. When several hundred lines are in question the amount of labor, thus multiplied, may seriously affect the numbers started. A scheme was accordingly devised whereby the selection of the desired virgin F_1 would be carried out automatically, by reason of the death, genetically produced before hatching, of all the undesired zygotes.

In the working of this scheme, advantage was taken of the peculiarities of the race containing "Translocation I," which had been discovered by BRIDGES to have a portion of one of its second chromosomes removed and attached to one of its third chromosomes. A zygote with a defective second chromosome cannot live unless supplied with the "translocated" section on the third chromosome. Thus, by making the undesired second chromosome of the ancestral female a defective one, there was a chance to kill off those offspring that received it, in a certain contingency—namely, when the translocated piece was not inherited with it. As for the other offspring that received this undesired second chromosome of the female, but received the translocated piece on the third chromosome as well, it was arranged to kill them in another way—by means of other lethals that had been placed in the third chromosomes. For this purpose, in the female in question, the third chromosome containing the translocated piece was given two different lethals (Δ and H), between which crossing over was prevented, and the two third chromosomes of the "source male" that was to be crossed with this female were each given one or the other of these same lethals. For these reasons none of the offspring survived that received the undesired second chromosome of the ancestral female. Now the desired second chromosome of this female contained the curly lethal complex. That made it easy to kill off those offspring that received this desired chromosome but received the undesired second chromosome from the "source male," for if the stock yielding the latter were made up in such a way that its undesired second chromosome contained the curly

lethal complex likewise, the offspring having the combination in question would be homozygous for curly and its associated lethal. Thus there would be left alive only the offspring of the requisite type that had received both the desired second chromosome from the female (the curly-containing chromosome) and also the desired second chromosome from the "source male" (the "test-chromosome").

The males and females serving for this cross were obtainable directly from their two respective stocks, without selection, as the ancestral combinations referred to were of a balanced type, such as would automatically perpetuate only their own composition (barring a very rare crossover). The formula of stock "A," from which the "source males" were derived, was as follows:

(stock "A") $$\frac{T^d\ b \qquad\qquad c_n\ c \qquad a_r\ m_r}{C_y\ l_{CyL}\ C_{CyL}\ c_n^2\ C_{CyR}} \quad \frac{\Delta}{H}$$

Δ ("delta") and H ("hairless") are the third-chromosome lethals previously referred to; they are dominant for certain visible characters, and their loci are very close together.

The formula of stock "B," from which the females for the cross were derived, is as follows:

(stock "B") $$\frac{P}{C_y\ l_{CyL}\ C_{CyL}\ c_n^2\ C_{CyR}} \quad \frac{\Delta He\ T_r}{C_{III}\ l_{III\,1}}$$

Here P ("Pale") represents the *absence* of the piece of the second chromosome which, when present attached to the third chromosome, is designated as T_r (for "translocation"). C_{III} is a "C factor"; $l_{III\,1}$ is a recessive "balancing" lethal; e ("ebony") is non-essential but would have been difficult to eliminate in making up the stock.

Reviewing this cross briefly now, in terms of the above symbols, we see first that the P-containing chromosome of stock "B" could survive only when accompanied by T_r. But T_r is accompanied by both the lethals Δ and H, either the one or the other of which it will also encounter from the other stock, "A," and which will therefore kill the fly containing it. The P chromosome, hence, never survives the cross. Considering now the curly-containing chromosome of stock "B," we see that this will, through its lethal, kill the zygote which also receives curly from "A." There still remains the possibility of curly from "B" (together with $C_{III}l_{III\,1}$) uniting with the "test-chromosome" from "A" (and with either Δ or H); this is the combination required; it has, so far as its second chromosomes are concerned, precisely the formula previously presented as desirable for flies of experimental balanced lethal lines.

To start the lines, then, it is accordingly necessary only to collect "A" males and virgin "B" females from the respective stock bottles, in large numbers, and then to put a pair in each vial, to be thrown out before the offspring hatch. Each pair is the ancestor of a separate line, or group of lines. Their offspring are automatically of the right composition, because of the genetic machinery that was at work, and those offspring that occur within a given culture all have their test chromosome derived from a single source-chromosome, and can be bred together.

AUTOMATIC PRELIMINARY TESTS OF THE LINES

Since, now, the test-chromosomes in the above offspring (F_1) do not intentionally contain star, or any other actual lethal, at the start, but only a sterilizing gene (m_r), the multiple recessives homozygous for this chromosome should actually appear, in the next generation (F_2) of the line of cultures, if this generation is reared under fairly favorably conditions (that is, in a bottle, in temperately warm surroundings). There they can be recognized through the glass wall of the bottle, with the unaided eye. An "antecedent" lethal unintentionally included will, however, prevent them from appearing. In this way, by merely growing the second generation with some care, in bottles, and inspecting the latter with the naked eye, the lines can be started with preliminary tests that will guarantee the absence of "antecedent lethals." The system based on the groupings of the lines will thus receive a double check, even "parallel mutations" can then be recognized as such, and the useless carrying along of lethal cultures through an experiment will be avoided.

It is true that the sterilizing gene morula cannot be regarded, theoretically, as completely preventing differential survival, because only the female is sterile. The flies containing all the recessive genes, however, hatch very infrequently in the vials, and then are small and weak. The number of lethals lost through the successful competition of a non-lethal homozygous multiple-recessive male of the type in question with a vigorous but lethal-bearing curly fly must therefore be negligible.

The development of the "automatic complementary stocks," "A" and "B," was the result of over a year's work (1921–1922), since many other schemes of mating and balancing, most of them much more elaborate genetically than the "A" and "B" plan described, were constructed and tried out simultaneously. Various others have also been tried since. The scheme given, then, represents the residue, proved most practicable, of all these various trials.

PROPAGATION OF THE CULTURES

The chief remaining portion of the work in which it was desirable to increase the productivity of a given amount of labor consisted in the sheer rearing of so great a number of cultures as was needed during the course of the "*n*" generations—the preparation of vials, emptying, transferring, etc. True, this work had, by the balanced lethal system, been reduced to a routine that allowed it to be placed in the hands of assistants, but the time consumed in such labor was so great as to leave the maximum number of lines that could be maintained smaller than was desirable, and much smaller than the number which the investigator, by means of the new simplifications in the crossing procedures, could conveniently establish and test.

Attempts were therefore made to improve the efficiency of the technique of propagation, and a number of features of considerable time-saving value were introduced, which it would scarcely be in place here to describe at length. Thus, a much readier method of distribution of the food among the vials, through a funnel, was introduced, and the food was made easier to handle, cheaper, and more suitable for the flies, by the substitution of an equal volume of 50 percent karo (aqueous solution) for half of the banana. The latter modification had already been introduced in the first experiment, and likewise the one of *spraying* the cultures *en masse*, with a yeast suspension, and of sprinkling them with confetti in place of inserting slips of paper. Such petty modifications as the substitution, for labels, of transferable tags attached to rubber bands, that were passed down from culture to culture in each line, also became of importance, when multiplied by the thousands of transfers made.

In spite of these and numerous other innovations, which, taken together, have about tripled the number of cultures possible, the problem of propagation of the lines still remains the most difficult one. It was for a time thought that this had been largely solved by the construction of concrete frames, in each of which 49 vials were embedded, and from which, after anaesthetization *en masse*, by cold, a single (fitted) cover, containing the flies in their respective pits, could be removed; all the flies of the 49 cultures at once might thus be transferred directly to 49 freshly prepared vials of culture medium, similarly embedded in another frame. Further experience has shown, however, that in occasional vials the flies will not yet be numerous enough for transferring, though all the others have long been ready; again, on some occasions, the flies may not fall into the cover pit in sufficient numbers. Thus more experimental work will be necessary

before we have a really feasible method of this general type. It is hoped that others interested in Drosophila cultivation will join in the endeavor to produce something practicable along these lines, as it might be of use also in the cultivation of stocks and in some other types of work.

FURTHER EXPERIMENTS

It is the object of the present paper to review only those experiments of the author, and that recorded work of others, which helped towards the ultimate attainment of an answer to the question whether temperature can somehow affect mutation rate. An account of the mutation work of the next three years, 1922–25, will, therefore, not be presented here. It did, however, play a rôle in the development of the final attack, as the considerable practice in balanced lethal work obtained then confirmed the practicability of using the automatic complementary stocks, "A" and "B," and in addition gave various important hints as to their use, which served to insure the carrying through of the later experiment. One such lesson drawn was that of the necessity of making the tests for lethals at a temperature not more than moderately high (about 25°C), and under not rapidly fluctuating degrees of humidity. Failure to have either one or the other of these conditions observed will lead to a very low viability of the multiple recessives that are sought for—a circumstance that caused the partial collapse of one extended experiment before these requirements were realized.

It may be explained that the reason that the work carried on by the writer, during these years, was in the main not directed at discovering the effect of temperature, was because much better physical facilities for such work were expected in 1925, with the completion of a new biology building containing a refrigeration plant, constant temperature room, and considerable incubator space. In the older building, it would not have been possible to insure the maintenance of nearly all the lines called for at one constant temperature and certainly not at two differing constant temperatures. Accordingly, in view of the amount of labor and expense that each mutation experiment entails, it was felt worth while to wait until the opportunities were optimal. Meanwhile, the experiments that were carried on were mainly directed at the related problem of the effect, on the mutation rate, of "age"—that is, of those internal conditions which change with the rotation of the reproductive cycle. The connection between this problem and our present one has previously been referred to. The results of these experiments, some of which are still in progress, must be published separately.

It should be mentioned that during this period one experiment involving temperature differences was undertaken. This was done, however, in collaboration with Professor F. B. HANSON, the cultures being carried on under his direction, with adequate facilities, at WASHINGTON UNIVERSITY. This experiment involved features which make it more suitable to report separately, or in connection with the experiments involving age differences. The omission of the results here will, it may be stated, in no way militate against the acceptance of the present data, or hamper their critical consideration, because in the joint work the temperature influence was applied in an entirely different way, and the results could not, and do not, either invalidate or confirm those of the present experiment. Meanwhile, the author wishes here to express his appreciation of Professor HANSON'S kindness in agreeing to this grouping of the reports.

THE LATEST TEMPERATURE EXPERIMENT

THE CONDUCT OF THE EXPERIMENT

With the approaching provision of the physical equipment needed, and attainment of facility with the improved breeding methods that had been evolved, it was decided to undertake an experiment which would considerably surpass, in numbers of chromosome-generations finally tested, any that had been previously performed, in order that, if it were at all possible, the question at issue might finally be settled decisively. At the same time it was desired to alter somewhat the method of application of the differential condition (temperature), so that certain more detailed information concerning the incidence of its effect (if any) might be secured.

In accordance with these plans, in May, 1925, 106 (fertile) pairs of flies, derived from the complementary stocks, "A" and "B," were placed in separate culture vials, to form the start of 106 groups of lines, numbered consecutively. In the next two generations, the descendants of each pair, without selection or etherization, were divided at random among 8 cultures, tagged with the given number of their original group, and lettered from A to H, respectively. In each group the appearance of some multiple recessive homozygotes, in some following generation, was noted; this proved that none of the groups of lines originally contained a lethal. The lines marked A to D were then chosen to form the "cooler" series, and those marked E to H, the series treated with a relatively high temperature.

It was intended to keep the cooler series continually at a temperature of 19°C, but as the new building was not ready as soon as expected these

cultures could not uniformly be kept as low as this for the first two generations following the separation of the two series. By dint of special efforts, however, it was managed to keep their temperature within about 2°C of this figure for this period, and after that, when they had been transferred to their new quarters, the temperature of these cultures varied, with rare exceptions, less than 1°C from 19°. Approximately ten days after the flies of this series had been transferred to fresh cultures, they were thrown out and discarded; a sufficient number of their offspring had, in the great majority of cases, hatched 10 or 11 days later (that is, 20–21 days after the parents had been put in), and these were then transferred to a fresh culture again, in which the cycle was repeated.

In April of the following year (1926) one male was taken from each of the surviving cooler lines, and crossed in the manner previously described (pp. 331-332), for the final tests for lethals.

The "warmer series" was subjected to a temperature of 27°C—care being taken not to allow it to go over a degree higher because, as WARD has found, this causes the curly complex to undergo appreciably more crossing over. The facilities were adequate, in both buildings, for maintaining the cultures at this temperature, ±1°. The "warmer series" of cultures were not kept at this temperature throughout their life cycle, however, as it was desired to test somewhat more specifically whether warmth, applied to that part of the life cycle more closely connected with the maturation period, would be effective in changing the rate of mutation. This was done in rather crude fashion in the first six generations of treatment (till the end of September, 1925), and with somewhat more refinement after that.

During the first six generations the cultures of the "warmer series" were kept with the cooler ones, at the same temperature as the latter, from the day on which the parent flies had been removed from them until, 10 days later, the offspring hatching in these cultures were transferred to fresh cultures. The latter were then kept at 27° for 10 days, until the flies (now parents) in it were removed, whereupon it likewise was placed in the cool environment and the cycle was repeated. This was not a critical method of applying warmth at a given period in the life cycle, because the flies, during their 10 days in the warm temperature, were laying eggs continuously, and both their egg cells and their offspring, including larvae of all possible different ages, must have been subjected to the warmth, which was accordingly applied, in the case of different individuals, to very different sections of the life cycle. During this portion of the experiment, then, the warmth may be regarded as having been

applied at practically all stages, but for only half the length of the life cycle. It will be noted, however, that nearly all the offspring were derived from eggs that had been subjected to warmth for at least a short time—generally a long time—before laying.

During the last 10 generations of treatment the warmth was again applied over about half (5/11) of the duration of the fly's life, but it was timed in such a way as to be limited somewhat more nearly to a certain portion of the life cycle, in each generation. This was done as follows: The parents were allowed to stay only four days in the culture from which the offspring were to be taken for continuance of the line. During this egg-laying period the cultures were given the 27° "treatment," and most of the eggs did not get beyond the young larval stage. After these four days the parents were discarded and the vial containing the larvae was placed in the cool room at 19°, with the other series. After about 12 days here those offspring which had by this time hatched into imagos were transferred to a fresh culture vial, which was put in the 27° incubator for 6 days. Then these same imagos (before their offspring had hatched) were retransferred into another culture for the period during which the eggs desired for the continuance of the line were laid; the preceding culture was discarded. Thereupon the cycle was repeated as before, keeping the new cultures containing the imagos (now parents) at 27° for four days, after which the imagos were discarded and the vial placed in the cool room, etc. It will be seen that in this case all the flies that bred had been kept at 27° for a period of 6 to 10 days just preceding the laying of the eggs that were allowed to develop. Aside from this period of warmth, which all the flies underwent, there was only the short period of warmth, 0–4 days long, during which the flies were in the egg and early larval stages. During their later larval development and growth, their pupal metamorphosis, and their earlier adult life, they lived at the same temperature as the cool series.

It will be noted that throughout the above series of breedings the warmer and the cooler series were carried through the same number of generations in a given time, and thus any possible influence of the chronological age of the parents was prevented. Of course the flies hatched sooner in the warmer series, but the time-length of the reproductive cycle that they were allowed to pass through was the same. For the flies of both series were transferred to those cultures from which their offspring were later to be taken, after the same length of time since their parents had been transferred to the cultures from which they themselves had hatched. In fact, these transfers were nearly always made on the same day, in both

series. In the first six generations, when double transfers of the imagos in the warm series were not made, the transfer of that series could not have been made two days later without a possible overlapping of generations, but tests showed that with the intervals and temperatures actually used overlapping did not occur.

It should be stated that in every experiment of this kind cultures occasionally die out, or occasionally they are not ready for transferring at the appointed time. Two cultures were therefore always kept of each line—the fresh culture and the one from which the flies had previously been transferred. If the fresh culture failed, flies were taken from the old culture wherever possible. In the case of the warm series, most of the flies in the older culture belonged to a "younger" generation than in the newer culture, not having been artificially retarded in their breeding by the transferring. Whenever an irregularity of this sort occurred, record was kept of it, so that the maximum total number of generations of the warm series, and the minimum of the cool series, could be computed, the figures presented in the next section, in order to be "on the side of caution" in interpreting the results, represent these extreme values. Such occurrences did not take place often, however, and could not have appreciably affected the mode of influence of the treatment upon the germ plasm of the organisms.

In April and May, 1926, one generation after the tests of the "cooler lines" had been started, the similar tests of the surviving "warmer lines" were begun.

THE RESULTS AND THEIR EVALUATION—THE EFFECTIVENESS OF TEMPERATURE WHEN THE DURATION OF THE GENERATION IS HELD CONSTANT

There were 381 lines of the original 424 belonging to the cooler series that had survived to be tested, representing 6286 "chromosome-generations" (*ln*), or 4191 "chromosome-months." Among these, only 12 lines, all of different groups, were found to contain a lethal; all others yielded the multiple recessives. This is a rate of 1 lethal in 524 chromosome-generations, or 0.19 percent of mutation per generation, for this chromosome. The time-rate is 1 in 349 chromosome-months, or 0.29 percent.

In the warmer series there were 359 surviving lines from the original 424, computed to represent 6462 "chromosome-generations," or 4308 chromosome-months. The number of these lines found to contain a lethal was 31. From these figures we may compute a mutation rate, per chromosome-

generation, of 1 in 208, or 0.48 percent. The time-rate is 1 in 139 chromosome-months, or 0.72 percent.

Of the lethals in the warm series only two sets, of two each, were numbered alike—that is, from the same group,—and only in three of the groups in which a lethal occurred in the warm series did one also occur in the cool series. This grouping of lethals was not greater than would be expected in a chance distribution; in fact, it was somewhat less than the most probable value for the expected grouping, though it did not deviate from the latter more widely than the error of random sampling would allow for. Every group containing one or more lethal lines also contained several surviving non-lethal lines. All lethals were verified by breeding the corresponding stocks derived from the test crosses through at least five generations, under favorable conditions, and examining the cultures in each generation for the multiple recessives. We may take the figure 31 of the warmer series as not too high, therefore, and as not representing the special mutability of a few groups of lines.

The difference between the mutation rates, per chromosome-generation, of the two series, is .29 percent. This is 4.1+ times its own probable error (calculated by the random sampling formula previously given). Such a difference would have only 1 chance of occurrence in 195 experiments, if the rates were really the same, or 1 in 390 if we consider the direction of the change as specified, and the effect may therefore be regarded as "proved," in the sense of having that high probability which passes as proof in most scientific work. The difference between the time rates of mutation is equally significant, since the time : generation relationship was the same in the two series.

Both the latest experiment, and the two previous temperature experiments combined, thus agree in giving convincing evidence that the *time-rate* of mutation varies with the temperature, the earlier results showing that this occurs when the duration of the generations varies naturally (more generations per unit time when warmer), and the recent results showing the same effect *even though* no more generations are allowed at the warmer temperature than at the cooler.

Regarding the results now from the point of view of the mutation rate per generation, it can be concluded from this last experiment that this rate rises when the temperature is increased, provided the absolute time-length of the generation be kept constant. Beyond this, from the earlier experiments, combined, there has been established a fair probability, though not as decisive as the above, that a rise in temperature increases the mutation rate, per generation, *even* when the generations

are allowed to have lengths that vary with the temperature as the rate of development naturally varies (the fly thus breeding at about the same "physiological age"). If this should hold true there would ordinarily be both more generations in a given time and, in addition, more mutations per generation, at a higher temperature, and the effect of temperature on the time-rate would therefore usually involve the product of both these factors.

It will be noticed that not only are the apparent effects on mutation rate of the same sign in all three experiments, as well as of convincing magnitude in certain cases considered separately, but that the intensity of the effect produced is also closely similar, approximating the relation, $Q_{10}=2$ to 3, well known for chemical reactions. Though not so much significance can be attached to the exact magnitude of the increment, nevertheless this agreement is "suggestive."

This apparently high effectiveness of heat was produced in the recent experiment even though the treated flies were, for the most part, subjected to the heat over only a special fraction of their life cycle—including principally the period of maturation or maturity, and to a lesser extent the period corresponding to the rather young larva. Only about half of the flies in about a third of the generations (the first six) were treated in other parts of their life cycle; the pronounced effects of heat observed could, therefore, scarcely all be referable to the relatively few treatments during these other periods. The indications are consequently very strong that heat at one or both of these two *particular periods*, just specified, is effective in increasing the mutation rate. Whether, when applied to other particular portions of the life cycle, the heat would be equally effective, or effective at all, we cannot say from such results, except by way of noting that the effects observed were as marked in the later experiment, when only this period was treated, as in the former, when the whole life cycle was treated. This problem, or a closely related one, is involved also in experiments that deal more specifically with the effect of aging.

INTERPRETATION OF THE FINDINGS, AND GENERAL CONSIDERATIONS

Although these results constitute, in the opinion of the writer, the first demonstration of the effectiveness of any specified agent whatever in influencing the mutations of numerous genes, and probably of genes in general, yet we must be exceptionally cautious in going far from these facts and attempting to draw still more general or remote conclusions from them. The sheer fact of the temperature effect on mutation

is worth having, but by itself it stands as an isolated beam in the largely unseen structure of mutation and gene theory. More results gained by similar methods are badly needed. But, in this connection, perhaps the most hopeful feature of the present data is that they show that mutation is indeed capable of being influenced "artificially"—that it does not stand as an unreachable god playing its pranks upon us from some impregnable citadel in the germ plasm; instead, it can be "moved," and its movements detected, studied and "mapped."

It should be repeated here that we do not as yet have any valid evidence on the question of how direct the effect of temperature upon mutation is. We know, however, that in the case of ordinary chemical reactions, the direct effect of a rise in temperature is in the direction of an increase in the speed of the processes, and that the magnitude of this increase is between about 100 and 200 percent for each 10 degrees centigrade (for ordinary temperatures). We have seen, in the present work, that in the case of mutation rate also the effect of a rise of temperature is in the "positive" direction, and that the magnitude of the effect observed here too seems rather simular to that just stated. These facts, then, certainly suggest that mutation depends primarily on a chemical reaction, and is thus directly affected by temperature; pushing the conclusion further, it would become probable that mutation consists ultimately in changes of structure of the general type conventionally designated as "chemical" rather than of one of the types called "physical," (not to speak of such imaginary types as vitalists might postulate). But these points can certainly not be regarded as critically proved, for changes in chemical reations, dependent on temperature, may in turn cause marked effects on physical processes, and *vice versa*.

We know, for example, that the frequency of the semi-mechanical process of crossing over is, in certain chromosome regions, about doubled by a rise of 10°C, at a certain temperature level, and this may quite possibly be brought about through a primary chemical effect of temperature, that in turn influences some "physical" property like chromosome plasticity. In some similar way it might be supposed that mutation, though itself a "physical" process (that is, not involving changes in intra-molecular attachments), could be influenced by a chemical change or complicated series of changes occurring outside of the genes. If a series of changes was involved, the initial process (the effect of temperature upon which was ultimately responsible for the changes in mutation frequency) might even be outside of the organism itself, since as has before been pointed out, we cannot absolutely exclude such,

possibilities as that a change in composition of the food, or in some other cultural condition, itself somehow dependent on the temperature, secondarily affected the mutation rate. We may return to the point, however, that in the case of any sorts of indirect action such as those pictured above, the facts that the effect of a rise in temperature was positive in direction, and was of apparently the same magnitude as are the direct effects on chemical reactions, would have to be regarded as in the nature of a "coincidence." And coincidences do not form good postulates.

One of the points to be remembered in considering the possible mode of action of any agent in changing the structure of a gene is that we are not necessarily dealing here simply with an alteration in the composition of pre-existing gene material, but we may instead, or in addition, be dealing with some kind of interference, by the agent in question, with the process by which the pre-existing gene forms new gene material. The pre-existing gene may remain unmutated, and the "mutation" may consist in the fact that, for some reason, the new gene material built up at that particular time was not just like the old. In that case, the more rapidly gene growth occurred (that is, the more gene material was formed in unit time) during the time that the "interfering agent" was able to act, the greater would be the number of mutations that occurred. Since an ordinary rise in temperature, during stages when cell growth is occurring, usually increases the rapidity of that growth, this by itself would then lead to a direct effect of temperature on mutation frequency, even if other effective factors remained constant. Evidence on this question might be gained if we found that the effect of temperature on mutation rate varied in direct proportion to the rapidity of gene growth that was going on in the germ plasm at the time when temperature was applied. For example, on this hypothesis, warmth applied to mature spermatozoa should produce no such effect. It was because of these considerations that the series of cultures involving the aging of spermatozoa was carried on, but, it will be recalled, the mutation rate was too low in that experiment to permit the securing of results. Similar work, with the aid of the newer methods, should be more informative.

There is at least one path of indirect action of temperature that might, on *a priori* grounds, have been postulated as a mechanism whereby mutation rate could be influenced, which can be categorically eliminated as a cause of the effects observed in the present experiments; that is, the possibility that the mutation rate was affected through the known effect of temperature upon crossing over. Such an idea may have already

suggested itself to the reader in view of the peculiar relationships found to exist between crossing over and bar eye "mutation," by STURTEVANT, on the one hand, and between crossing over and reddish mutation in *D. virilis*, by DEMEREC, on the other hand. Fortunately for a decision on this point, crossing over could not occur in the present experiments, in those chromosomes in which mutation was looked for. Furthermore, most of the mutations occurred in chromosome regions the crossover frequency of which is affected little or not at all by ordinary temperature changes. The mutations observed, then, were not phenomena of exchange between homologous chromosomes. They may well have been affected, however, by some of the same forces (for example, those exerted in synaptic attraction) as also influence the process of crossing over.

An attack on such questions as the above, also, does not now seem so remote. Whether or not, or how, certain synaptic occurrences are associated with mutational changes in general is one of the topics that may be investigated by modifications of our present methods. An intensive mutation study, in which given conditions, known to affect another process in question (for example, synapsis), are concentrated at crucial stages of the life cycle, could scarcely fail to yield evidence regarding such a point.

In the light of the new "gene-element" conception further and perhaps even deeper problems are raised by the present study. Accepting, that is, for purposes of discussion, this new theory, it is not clear whether the effect of temperature here detected would be due to a greater rate of sorting out of "gene-elements" already heterogeneous in the gene before the experiment started, or to an actually greater rate of "gene-element mutation," or both. Various indications, however, would point to the rate of mutation in whatever are the primary gene particles as having itself been increased. One of these indications is the usual lack of grouping in sister lines of those identically located lethals which were found in most of the experiments,—although the latest experiment on the X here seems to form an exception. Another indication is the finding of apparently as great an effect of temperature on mutation frequency, per generation, in the experiments involving many generations as in those involving few, whereas if merely the rate of sorting out of elements had been hastened the supply of differing elements would have dwindled away in the course of time. On this point too, however, the results are only "suggestive." The methods here used are, however, capable of application to this problem too.

Evolution theory and practical breeding must in part follow in the

wake of mutation study. Evolutionists would doubtless eagerly make use of the notion that mutation happens more frequently, per unit time, and also probably per natural generation, at warmer temperatures. And if this were constantly true it could scarcely fail to be an important factor in the rate of evolution, since mutations seem to be so rare that their rate may often be the *limiting* factor in the rate of evolution, and the latter process will then be directly proportional to the former (other things being equal). But it must be pointed out that the significance of the results here presented, for evolution, must largely depend upon the answer to some of the problems previously raised. Take, for example, the problem last discussed, as to which hypothetical part of the process of mutation has been accelerated: The change in the ultimate gene elements, or in their postulated rate of sorting out (and, possibly, in their differential rate of multiplication) to form manifestly different genes. If the former process has been speeded up, the effect would indeed be important in the long run, and therefore of consequence in evolution; if the lattter process only is involved, the effect might be evanescent, since the rate of supply of new "gene-elements" would not be increased. It must be remembered, too, that "other things" are not equal, in nature, and that other factors (including those of selection) differentiating warmer from cooler climates may, on occasion, be vastly more influential than an effect of temperature upon mutation rate itself. This too, however, is within the pale of investigation.

In practical breeding, any factor should be of importance that can affect mutation rate, even if for only a few generations. The implications of the present study for the improvement of organisms whose germ cells can be subjected to controlled temperatures are therefore obvious, and need not be dwelt upon here further. Conversely, cold might be used as an aid in maintaining genic stability in already standardized races.

In conclusion, it may be repeated that, while the effect of temperature on mutation here observed seems of interest, more special emphasis should perhaps be placed on the opening up of the new methods here set forth, and on the proof that these methods can be used successfully in attacking problems which hitherto have been inaccessible. Temperature is merely one of a great number of conditions—external and internal—the effect of which upon mutation can be studied in various ways. And, as the present work demonstrates, some of these other conditions, or at least one, certainly do exert an influence upon mutation rate far greater even than that of temperature. As to what these conditions, or this condition, consist of, the present experiments give little hint, though

they varied markedly from experiment to experiment. This in itself presents an alluring problem, which likewise seems capable of approach through the present methods. Thus, through attacks of this kind, we may perhaps hope for the study of mutation eventually to pass from its earlier observational and speculative stage to one of quantitative and controlled study, from which exact knowledge, and principles not now to be guessed, may finally emerge. The "factor theory" itself awaited intensive quantitative study before its structure could be soundly established, so did the chromosome theory, and so, probably, will the future theory of mutation.

It may appear as though experiments of this type are too cumbersome to be prosecuted. They are not nearly so cumbersome as they were when the first results were obtained with them, and still better methods are, it is hoped, being developed. Since, however, the methods can be successfully used at all, then, for the very reason that they do require effort, it becomes all the more needful for a larger corps of investigators to step into the work thus provided, to make still further improvements, and to gain further data on the important problems that abound in this new field. Each plant-generation in the earlier work on Mendelian inheritance required a year, and much labor, and an experiment required several years; yet through such work biology made relatively rapid strides. And the mutation work is now only in its early years.

SUMMARY

1. The development is traced of methods of obtaining valid data on the frequency of gene mutations under varying conditions. The methods fall under two general groups:

(a) Tests of the X-chromosomes. These again fall into two subgroups—those involving sex ratio counts of each test-culture, and those involving the determination of the presence or absence, in each test-culture, of certain classes of males from mothers heterozygous for sex-linked "identifying genes."

(b) Tests of autosomes. Here stocks containing balanced lethal or sterility genes may be used, to allow the accumulation of mutant genes with the exclusion of natural selection, and the tests, involving "identifying characters" observed in the second generation, are then applied only to the final test-culture of each of the numerous lines of descent.

2. Explanations are given of the purposes, and of the modes of operation of various special genetic devices that facilitate the establishment of the test-cultures and the making of the tests, so as to render possible the

obtaining of a significantly large body of data. Among these devices may be mentioned particularly:

(a) In the case of work on the X-chromosome, the use of the "C_l" complex, discovered in the course of the work, which makes it possible to detect sex-linked lethals by inspection of the culture vessels with the naked eye, to use vials instead of bottles as culture vessels, and to continue the tests in each generation by simply crossing non-virgin females with any of their brothers.

(b) In tests on the second chromosome, the use of the "curly" complex in the "balancing chromosome," in conjunction with a sterility gene instead of a lethal in the test-chromosome; this likewise makes possible the detection of lethals by naked eye inspection of the culture vessels.

(c) In establishing the initial cultures in work with the second chromosome, the use of "translocation I," together with lethals in the third chromosomes, in special arrangement, to kill off automatically all flies except those of the required composition and thus to insure the "unity of the source" of tested chromatin in each line, or group of lines, of descent.

(d) The method of grouping the balanced lines in such a way that lethals present prior to the beginning of the experiment would be recognized as such when the final tests were made, and the splitting of each group of lines between the two contrasted series of an experiment so as to avoid the effects of invisible genetic heterogeneity.

3. The tests carried out by the aid of these methods demonstrated their adequacy, under "suitable conditions" (see 5), for the attainment of significant figures, in which the error caused by "random sampling" was sufficiently smaller than differences due to "determinate causes." This will be realized on inspection of table 3, pp. 354–5, where the data from all the experiments herein reported are summarized,—comprising a total of over twenty-four thousand definitive test-cultures and a still greater number of chromosomes tested for mutation.

4. The lethals found in some of the experiments were mapped (see figures 1 and 2), and tests to determine allelomorphism were given. While a few more mutable loci were encountered, on the whole the genes were distributed in a manner similar to that found in ordinary work on "visible genes." Gradations between lethals and "visible genes" were found, as well as cases of allelomorphism between lethals and visibles. Thus, detailed study of the lethals has shown that they do not constitute a class genetically different from other mutants, or confined to a few

special loci, but that the mutations giving rise to them may legitimately be used as an index of gene mutations in general.

5. The results obtained in different experiments show beyond question that, instead of mutation proceeding at a fixed rate, as might have been supposed, it is exceedingly changeable in its frequency, and, in fact, variations of the order of 1000 percent can follow from unknown causes that invisibly differentiate experiments apparently similar, and involving only ordinary cultural conditions. In the light of these findings, it is probable that the failure of attempts of previous investigators to prove any effect of drastic external conditions upon the occurrence of mutation has been due rather to lack of refinement of their methods (which utilized only the exceedingly rare definitely "visible" mutations as an index of mutation rate) than to actual non-effectiveness of any of the agents employed.

6. It is found that, at the higher levels of mutation frequency encountered in the course of these experiments, it is practicable to use the X-chromosome in studies of mutation rate, but, at the lower levels, such as were more often encountered, the most highly improved methods involving balanced stocks are usually desirable.

7. Results from the earlier temperature experiments on both the X and the second chromosome agree in rendering it fairly probable, though by no means certain (since genetic heterogeneity was not so well allowed for) that the rate of mutation, *per generation*, rises with increase in temperature, even though the generations at the higher temperature are allowed to occupy a correspondingly shorter time (the generations at both temperatures being allowed to succeed one another at rates about proportional to their maximum rates for those temperatures).

8. The experiments just mentioned indicate, with a much higher probability, that the frequency of mutations, in a given time,—that is, the *time-rate* of mutation—rises with increase in temperature, when the generations thus succeed each other at the rates "natural" for the respective temperatures.

9. The latest experiment on the second chromosome, employing improved methods that made possible the use of a much larger number of cultures and also the definitive elimination of both genetic and cultural heterogeneity as possible agents in a differential effect, yielded decisive evidence of a rise in mutation rate, dependent upon increase of temperature, when the duration of the generations was caused to be the same at the two temperatures. Under these circumstances, then, both the time-

rate of mutation, and the rate per generation, is known to be affected by temperature.

10. During the greater part of the time during which the experiment just referred to was carried on, the flies of the "warmer series" were kept at the higher temperature only for the later portion of their imaginal life (for 6 to 10 days) and in relatively early larval stages (for 0 to 4 days); for the rest of their lives (for 11 days) they remained at the same temperature as that at which the "cooler series" was kept constantly. It is therefore likely that warmth is effective in influencing mutation when applied specifically to the stages mentioned.

11. *It should be noted that both the direction of the effect of temperature* on the time-rate of mutation, and its approximate magnitude, are the same as in the case of its effect on the time-rate of ordinary chemical reactions.

12. Possible interpretations of the findings concerning temperature are discussed, and their bearings on other topics are pointed out.

13. It is believed that the methods by which these results have been obtained open up a new field of genetics—the quantitative study of gene mutation, as occurring throughout one or more entire chromosomes under purposely varied conditions.

TABLE 1

Distribution of cultures in the 1921–1922 mutation experiment on the X-chromosome.

NUMERICAL DESIGNATION OF P_1 PAIR	STOCK OF P_1 MALE	NUMBER OF P_2 PAIRS FROM CORRESPONDING P_1 PAIR	NUMBER OF LETHALS IN P_2	NUMBER OF P_3 PAIRS FROM CORRESPONDING P_2 PAIRS	NUMBER OF LETHALS IN P_3	NUMBER OF P_4 PAIRS FROM CORRESPONDING P_3 PAIRS	NUMBER OF LETHALS IN P_4
1	Fla.	36		32	3	19	1
2	"	19		16		9	
3	"	45		36		22	
4	"	5		5		2	
5	"	33		24		23	
6	"	6		5		2	
7	"	60	2	50		31	1
8	"	7		5		3	
9	"	27		23		15	
10	"	50		48		39	
11	"	27		26		22	
12	"	6		4		4	
13	"	19		16		10	
Total Florida		340	2	290	3	201	2
14	Falm.	8	1	4		2	
15	"	10		10		8	
16	"	26		25		22	
17	"	30		27		17	
18	"	30		29		26	
19	"	30		28		17	
20	"	17		17		11	
21	"	9		8		5	
22	"	4		4		3	
23	"	10		9		6	
24	"	46		45		43	
25	"	23	1	22		19	
26	"	24		24		23	
Total Falmouth		267	2	252	0	202	0
27	C_{IA}	49		43	1	23	
28	"	22		19		11	
Grand Total		678	4	604	4	437	2

TABLE 2

Lethals found in the 1921-1922 mutation experiment on the X-chromesome.

DERIVATION OF LETHAL-CONTAINING CULTURE[1]	GENERATION OF FEMALE FOUND HETEROZYGOUS FOR THE LETHAL	APPROXIMATE LOCATION OF THE LETHAL	CLASSIFICATION OF CHROMOSOME IN WHICH LETHAL AROSE
1.7	P_4	near *w*^{e}	Paternal; recessive
1.8	P_3	slightly right of *w*^{e}	Paternal; recessive
1.27	P_3	near *W*	Maternal; dominant
1.28	P_3	slightly left of *V*	Maternal; dominant
7.4	P_2	very near *W*	Paternal; dominant
7.27	P_2	very near *W*	Paternal; dominant
7.34	P_4	slightly right of *W*	Maternal; dominant
14.1	P_2	near *v*	Maternal; recessive
25.13	P_2	slightly left of *F*	Paternal; dominant
27.26	P_3	near *w*^{e}	Paternal; recessive

[1] First number given is that of ancestral P_1 pair; second number denotes which P_2 pair from that P_1 pair the lethal culture consisted of, or was derived from.

TABLE 3

Chronological summary of mutation experiments reported in the present paper.

	EXPERIMENTERS	TIME OF EXPERIMENT (EXCLUSIVE OF PREPARATION OF STOCKS, MAPPING OF LETHALS, ETC.)	PLACE OF EXPERIMENT	CHROMOSOME TESTED	TYPE OF CULTURES	APPROXIMATE TEMPERATURES	TOTAL NUMBER OF "TEST CULTURES" (EXCLUSIVE OF PRELIMINARIES ETC.)
1	Muller with class	1918 (spring)	Houston, Texas	X	heterozygous *w^e v f*	warm, room (25°±)	results indefinite (100 to 200)
2	Altenburg	1918–1919 (winter and spring)	Houston, Texas	X	wild type (sex ratio tests)	warm, room (25°±)	385
3	Altenburg and Muller	1919 (summer)	Woods Hole, Mass.	X	heterozygous *w^e v f*	warm(27.5°)	517
						moderate, room	(100)
						cooler (19.5°±)	445
4	Muller	1919 (spring) to 1920 (fall)	New York City and Austin, Texas	second	balanced lethal	warm (26.5°)	4098
						cooler (18°±)	726
5	Altenburg and Muller	1920 (summer)	Woods Hole, Mass.	X	complex heterozygous	warm (27.5°) and cooler (21°±)	results indefinite (2000+)
6	Muller	1921 (winter and spring)	Austin, Texas	X	involving "C_l" inversion	warm (27°)	3935
7	Muller	1921–1922 (fall and winter)	"	X	heterozygous *w^a v f*	warm (26.5°)	1719
8	Muller	1925 (spring) to 1926 (spring)	"	second	modified balanced lethal	warm 27°)	6462
						cool (19°)	6286
9	Total of definite counts						24,573

TABLE 3 (Continued)

	NUMBER OF CHROMOSOMES TESTED PER CULTURE	NUMBER OF CHROMOSOME GENERATIONS TESTED	NUMBER OF LETHALS FOUND	NUMBER OF CHROMOSOME-GENERATIONS INCLUDING ONE LETHAL	NUMBER OF CHROMOSOME-MONTHS INCLUDING ONE LETHAL	DIFFERENCE BETWEEN TIME-RATES OF MUTATION IN WARMER AND COOLER SERIES ÷ ITS PROBABLE ERROR	RESULTS REPORTED ON PAGES
1	2		(1)				307
2	2	770	13	59	30		309
3	2	1034	13	80	34	3.0	311-314
	(1)	(100)	(2)				
	2	890	5	178	94		
4	1	4098	24	170	85	2.9	301-302
	1	726	2	363	323		305-306
5	2		"numerous" but indefinite				318
6	1	3935	4	984	430		321-322
7	2	3438	10	344	155		324-326
8	1	6462	31	208	139	4.1	341-342
	1	6286	12	524	349		
9		27,639	114	(242)	(138)		

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MUTABLE CHARACTERS OF *DROSOPHILA VIRILIS*

I. REDDISH-ALPHA BODY CHARACTER *

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INTRODUCTION

In a preliminary report on the behavior of the "reddish" body-color character (DEMEREC 1926a) it was suggested that the unusual results obtained in different experiments could readily be explained by the assumption that reddish mutates frequently to the wild-type. This assumption has been made still more probable by the results obtained in recent studies on reddish. It is also supported by the work on the behavior of the miniature-α wing character (DEMEREC 1926b) and the magenta-α eye-color character (DEMEREC 1927a), both of which revert

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in the same way as reddish does. In this paper an endeavor will be made to give a detailed account of the results obtained only in experiments with reddish. The details of the experiments with miniature-α and magenta-α will be presented in subsequent papers of the same series.

NOMENCLATURE

To designate a character showing frequent heritable changes, the terms "mutable", or "frequently mutating" will be used. The process of change will be called "mutation", and also "reversion"; since in the case of reddish it was observed that the change occurs only in one direction, namely from reddish to wild-type. Thus the term "mutation" and its derivatives are applied both to the reversion of a mutant character to the wild-type, as well as to the origin of the mutant character from the wild-type.

The Greek alphabet is used to distinguish mutable from constant allelomorphs.

The symbol *re*-$\propto$ will be applied to the mutable reddish. Since reddish is an allelomorph to yellow, and yellow has been known longer (METZ 1916) than reddish the strictly correct symbol for the mutable reddish would be $y^{re\text{-}\alpha}$. As a matter of convenience, however, the shortened symbol *re*-α will be used in this paper.

THE ORIGIN OF REDDISH

The character called reddish was found twice in matings involving in both cases the stock line, concave, approximated, telescoped. The period of seventeen months elapsed between the first and the second origin. The first reddish is mutable; and will be called reddish-α while the second reddish is constant, and will be called reddish-1. The second reddish was found by Miss MILDRED S. MOSES, to whom the writer is indebted for permission to use the data relating to the origin of reddish-1 and also for the use of flies bearing that character in experiments.

The origin of reddish-alpha

In experiments planned to test the effect of centrifugal force on the behavior of sex chromosomes, a cross was made in which a centrifuged yellow vermilion female was crossed with a sepia scute short rough male; and among a total of 104 offspring, a female was found which had several extra veins on both wings. To determine the inheritance of this character, the offspring of the female were tested for two generations. When it was found to be a dominant autosomal character, a cross was made to

determine the chromosome in which it was located. For that purpose, a concave approximated telescoped female was crossed with a sepia vermilion branched-veins male; and five of the F_1 females were backcrossed with concave approximated telescoped males. In one of the backcrosses, half of the males had a reddish body; and in another backcross, among a total of 217 flies, one reddish male was found. The remaining three backcrosses gave no reddish flies.

Subsequent tests showed that the extra-veins character was a recurrence of branched, the latter having been already described (METZ, MOSES and MASON 1923). Reddish flies, therefore, originated from the cross between a newly found branched and concave approximated telescoped flies. They were found on January 8, 1924, in two separate matings. In one case, half of the males were reddish; indicating that the female parent (P_1) was heterozygous for the character. It is possible that in this case the mutation occurred late in the P_2 female, affecting only a few germ cells, one of which was transmitted to the P_1 female; or that the mutation occurred early in the development of the P_1 female, making it heterozygous for reddish; or that the mutation affected a few P_2 males. In the second mating noted above, where only one reddish male was found among 217 flies, the possibility is not excluded that it came as a contamination from the other mating, where many such males were found. It is, however, at least as probable that it originated as an independent mutation, affecting only a few germ cells of the P_1 female, as that it came as a contamination.

All reddish flies used in the experiments were progenies of the mating which gave half of the males reddish. The single reddish male found in the other mating was not used in breeding.

The origin of reddish-1

To locate a certain newly found character, which turned out to be another allelomorph of branched, Miss M. MOSES made a cross between the character in question and concave approximated telescoped flies from the stock culture. On June 16, 1925, in one of two backcrosses, one reddish male was found among a total of 145 flies. Reddish-1, therefore, originated, similarly to reddish-α, in a backcross involving the newly found character branched and concave approximated telescoped.

Breeding experiments with the original reddish-1 male showed that it was heterozygous for the characters concave approximated and telescoped. Since there were no reddish-α flies which could have had a

similar genetic constitution, at that time in the laboratory, it is unlikely that the first reddish-1 male was a contamination from reddish-α. The results of crosses between reddish-α and reddish-1, which will be discussed later, made the possibility of contamination having occurred still more improbable.

Attempts to obtain other reddish mutants

Both reddish-α and reddish-1, as already stated, originated in matings involving branched and concave approximated telescoped characters. The character, branched, used in these different crosses, came from unrelated sources; but the concave approximated telescoped flies were in both cases taken from the same stock line. It is probable that it was due to chance that both of the reddish mutations occurred in the progenies of crosses where flies from a particular line were used; but there is a possibility that the line concave, approximated telescoped, used in both cases, was potentially mutable to reddish. To test that possibility, the cross between concave approximated telescoped and branched flies was repeated seven times; and 217 F_1 females from these crosses were backcrossed with concave approximated telescoped males. Among 16,890 offspring of these backcrosses no reddish flies were found. These results indicate that if the concave approximated telescoped line mutates to reddish at all, the frequency of such mutations is very low.

DESCRIPTION

The body, wings, legs, bristles and hairs of the reddish flies are reddish-yellow; and contrast with the dark gray color of these parts of the body in wild-type flies, and the yellowish gray color in yellow flies. The color of the flies varies with the age and condition of the cultures. Extreme variations of reddish and yellow might overlap in color of the body; but no case has been observed where reddish and yellow could not be distinguished by the color of the hairs and bristles, which is yellowish gray in reddish and gray in the yellow flies.

Reddish-α and reddish-1 can not be distinguished.

PARTIAL MAP OF THE SEX CHROMOSOME

Several sex-linked characters were extensively used in the experiments which will be discussed in this paper. For a better understanding of the data the partial map of the X-chromosome given in figure 1 may be useful. The majority of the characters mentioned in the map have already been described (METZ, MOSES AND MASON 1923). The descriptions

of some of them, however, are not yet published, since the characters were only recently found by either Dr. C. W. METZ or Miss MILDRED S. MOSES, to whom the writer is indebted for the use of the material.

INHERITANCE OF REDDISH-ALPHA

Reddish-α was found to be a sex-linked recessive character. The crosses between reddish females and not-reddish males always gave not-reddish females and reddish males; and from the matings where not-reddish females and reddish males were used, all the F_1 generation was not-reddish.

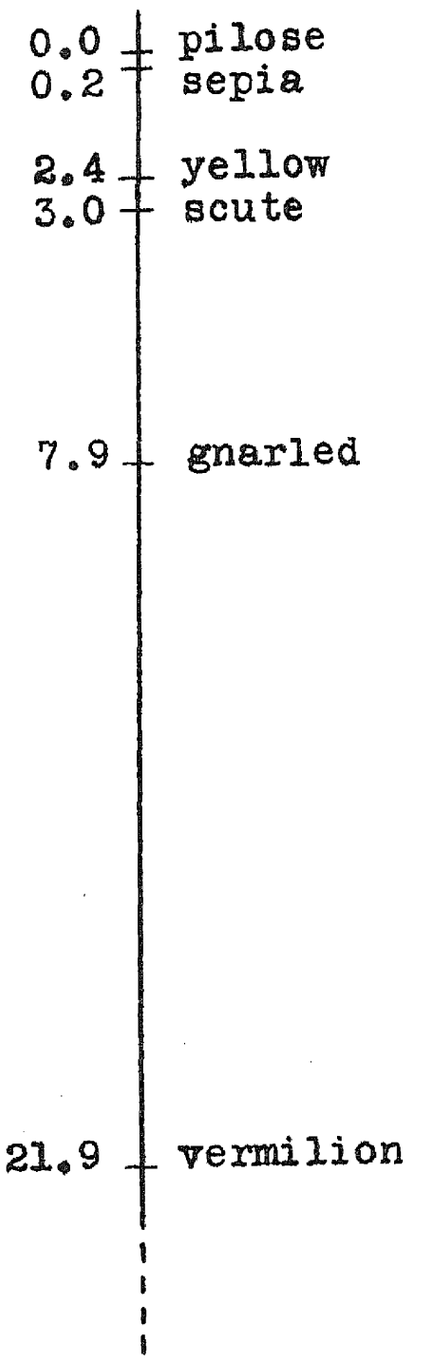


FIGURE 1.—Map of left end of sex-chromosome.

Allelomorphism to yellow

If two allelomorphs are recessive to wild-type, the F_1 generation when they are crossed is usually intermediate. It could not be of the wild-type, since it is assumed that allelomorphs are located in homologous loci, and only two of them can be present in the usual type of inheritance. Two non-allelomorphic recessive characters on the other hand, always give a wild-type F_1 generation. In the F_2 generation of the crosses between two allelomorphs, only parental and F_1 types are expected. Bar in *Drosophila melanogaster* (STURTEVANT 1925) is the only case where two allelomorphs combine in one chromosome, and that rarely. It has often been pointed out that, in all cases where several allelomorphs are known, they affect the same character. The difference between them is rather quantitative than qualitative; therefore the test for allelomorphs should show (a) that the F_1 generation from a cross between two of them is not of the wild-type; (b) that they do not recombine in the F_2 generation (c) that their position in respect to other genes is the same (except in case of such chromosomal abnor-

malities as duplication or inversion), and (d) also that they affect the same character.

Reddish and yellow fulfill these requirements. When intercrossed,

TABLE 1

Data from the crosses with "constant" reddish-α, showing linkage relations.

CONSTITUTION OF F_1 ♀'s			r_e / $s_e y\ s_c v$	r_e / $pl\ s_c$				$s_e r_c$ / $pl\ s_c$	$pl r_c$ / $s_e y\ v_n$	$r_e v_n$ / $s_e y\ s_c v$		
				1st experiment	2nd experiment	3rd experiment	Total			1st experiment	2nd experiment	Total
F_1 pedigree number			969	505	710	711	..	966	967	968	970	..
No. of F_2 matings			23	6	10	7	23	15	16	22	14	36
Total ♀			1972	500	434	301	1235	1222	1349	1812	978	2790
♂	0*	a†	849	268	173	137	578	533	530	610	274	884
		b	664	207	134	86	427	593	471	537	347	884
	1	a	26	4	1	1	6	2	1	17	7	24
		b	22	1	4	3	8	1	..	13	12	25
	2	a	6	6	1	..	7	18	6	2	1	3
		b	7	3	2	..	5	10	4	3	1	4
	3 -	a	193	..	..	..	..	1	21	43	19	62
		b	151	..	..	..	..	6	33	36	13	49
	4	a	..	..	..	..	..	..	..	103	48	151
		b	..	..	..	..	..	..	..	113	49	162
	1–3	a	..	..	..	..	..	..	..	..	..	..
		b	1	..	..	..	..	..	..	..	..	1
	1–4	a	..	..	..	..	..	..	..	1	..	1
		b	..	..	..	..	..	..	..	1	1	2
	Total		1919	489	315	227	1031	1164	1066	1479	772	2251

* In this and the following tables "0" signifies non-crossover classes; "1" classes resulting from crossing over in the first region, etc.

† The class having r_e is always placed in column *a*.

F_1 females were always yellow or nearly so. Since the color of the body of the flies varies with age and environmental conditions, it is difficult to detect slight differences due to the genetic constitution; and it is not

TABLE 2

Data from the crosses $r_e/s_e\ y\ s_c\ v$ ♀ × $s_e\ y\ s_c\ v$ ♂.

Number of experiments			32
Number of matings			184
	r_e and y		11053
♀'s	Reversions	+	138
		v	22
		$v\ s_c$	18
	Total		11231
♂'s	0	r_e	4067
		$s_e\ y\ s_c\ v$	3648
	1	$s_e\ r_e$	92
		$y\ s_c\ v$	75
	2	$r_e\ s_c\ v$	25
		$s_e\ y$	37
	3	$r_e\ v$	984
		$s_e\ y\ s_c$	885
	1–3	$s_e\ r_e\ v$	..
		$y\ s_c$	3
	2–3	$r_e\ s_c$	1
		$s_e\ y\ v$	..
	Reversions	+	118
		v	30
		s_c	23
	Total		9988
Total	y and r_e		20870
	Reversions		349

possible to determine with certainty if reddish yellow females are not lighter than homozygous yellow females. If there is any difference between these two types of females it is very slight.

Soon after its discovery, two lines of reddish-α were isolated: one which mutated frequently to wild-type; and one in which reddish-α behaved like a constant character. In the F_2 generations from the crosses between yellow and "constant" reddish-α, only reddish and yellow offspring were obtained; as was to be expected, if these two characters were allelomorphs. In table 1, 11,347 progeny from such crosses are listed. Table 1 gives

TABLE 3

Data from the crosses pl re/s_e y s_e ♀ × s_ey s_e ♂.

Number of experiments			12
Number of matings			98
♀'s	r_e and y		5462
	Reversions	+	68
		s_e	5
		s_e	1
	Total		5536
♂'s	0	r_e p_l	2119
		s_e y s_e	2299
	1	r_e	..
		p_l s_e y s_e	2
	2	s_e r_e	57
		p_l y s_e	37
	3	p_l r_e s_e	12
		s_e y	29
	Reversions	p_l	51
		p_l s_e	5
	Total		4611
Total	r_e and y		10017
	Reversions		130

the data from crosses involving constant reddish-α and pilose, sepia, yellow, scute, gnarled and vermilion, which indicate that reddish-α is located in approximately the same region of the chromosome as yellow.

Finally both yellow and reddish-α are similar in effect, as is the case, as already stated, with all known allelomorphs belonging to one group.

This evidence supports the assumption that reddish-α is an allelomorph of yellow.

Mutability of reddish-alpha

There is, however, a line of reddish-α which does not behave like the line described in the previous section. When reddish from that line is crossed with yellow, F_1 females are yellow, as usual; but in the F_2 generation, in addition to reddish and yellow flies, wild-type flies appear in a variable number. If reddish is an allelomorph of yellow, as the analysis of the data from table 1 indicates, then these wild-type flies are exceptions from the usual behavior of allelomorphs. Or the wild-type flies obtained in crosses between reddish-α and yellow might be crossovers between these two factors; which would indicate that reddish and yellow are not allelomorphs, and that the composition of the F_1 generation in crosses between them was exceptional.

TABLE 4

Data from the crosses $r_e/pl\ s_c$ ♀ × $pl\ s_c$ ♂.

NUMBER OF EXPERIMENTS	NUMBER OF MATINGS	TOTAL ♀	♂							TOTAL
			0		1		2		Reversions	
			r_e	$pl\ s_c$	$r_e\ pl$	s_c	$r_e\ s_c$	pl	+	
6	75	5207	2196	1978	31	50	17	27	150	4449

In tables 2, 3 and 4 a summary is given of the data from crosses involving reddish-α and pilose, sepia, yellow, scute and vermilion. From table 2, which summarizes crosses between reddish-α and sepia yellow scute vermilion, it can be seen that the not-reddish and not-yellow classes (listed under the heading of reversions) offer an obstacle to the usual analysis of the data. As already pointed out in an earlier paper (DEMEREC 1926); if all classes are considered together, it is not possible to locate reddish in the region of the chromosome where it belongs. When an analysis is made in that way, reddish does not fit into the linear arrangement of the genes. It is not probable that crossing over is responsible for the occurrence of these not-reddish and not-yellow classes; since double crossovers between the sepia and scute regions would be required to obtain the observed combinations. The distance between sepia and scute is about 3 units, which is much too short to allow any double crossovers, especially as many as there would be if the not-reddish and not-yellow flies were assumed to be double crossovers. The same holds true when the data given in tables 3 and 4 are analysed.

The problem looks much simpler if the analysis is made without the

not-reddish and not-yellow classes. Then reddish behaves in the same way as in the crosses discussed in the previous chapters; that is, as an allelomorph of yellow. In that case, however, it is necessary to account for the origin of the not-reddish and not-yellow classes. An examination of the data given in the columns headed "reversions" in tables 2, 3 and 4, reveals that all these classes have the reddish chromosome, in which the wild-type allelomorph has been substituted for reddish. As already indicated, crossingover can not account for that substitution. The only other probable assumption is that in these cases reddish mutated back to the wild-type allelomorph. That assumption would easily account for the origin of all the observed classes. If it is assumed that reddish is an allelomorph of yellow and frequently reverts to wild-type, then all

TABLE 5

Comparison between reddish and not-reddish classes in crosses with constant and mutable reddish-α.

CROSS	MUTABLE REDDISH						CONSTANT REDDISH			
	r_e		Not r_e		Reversions		r_e		Not r_e	
	Total	Percent	Total	Percent	Total	Percent	Total	Percent	Total	Percent
$r_e \times y\ v$	223	40.2	251	45.2	81	14.6	479	53.0	424	47.0
$r_e \times p_l\ s_c$	2062	50.0	1911	46.4	149	3.6	769	56.6	589	43.4
$r_e \times s_e\ y\ s_c\ v$	5024	51.5	4551	46.6	186	1.9	3214	52.8	2877	47.2
$r_e\ p_l \times s_e\ y\ s_e\ v$	2019	47.2	2203	51.6	51	1.2	551	48.6	583	51.4

wild-type classes from crosses between reddish and sepia yellow scute vermilion could be interpreted as reversions. That would separate them from the remaining classes, and eliminate all the difficulties otherwise encountered in the explanation of the results.

If reddish-α reverts frequently to the wild-type allelomorph, it ought to be expected that, in the experiments involving mutable reddish-α the reddish classes would be in deficiency when compared with the not-reddish classes. Morever, it is to be expected that the deficiency would correspond to the number of reversions. Unfortunately there is a differental viability between reddish and yellow flies, which is still more pronounced if pilose or sepia and scute are present. That prevents a direct comparison between the reddish and not-reddish classes. When, however, the reddish classes from the experiments with mutable reddish are compared with the reddish classes from experiments with constant reddish (table 5), and both of them are compared with the not-reddish classes of the same experiments, it is noticeable that the mutable reddish shows a deficit approximately equal to the number of reversions.

Constancy of reverted-reddish-alpha

To test the behavior of the wild-type flies which originated as reversions from the mutable reddish-α, 42 wild-type males were mated to sepia yellow scute vermilion females. All F_1 females were wild-type, and of 18,811 F_2 offspring, none was reddish. These results indicate that the mutations in the reddish locus occur in one direction only, namely, from reddish to wild-type, and not from wild-type to reddish.

TABLE 6

Distribution of reversions among the progenies of single females which females came from a pair mating.

PEDIGREE NUMBER	TOTAL NUMBER OF FLIES	NUMBER OF REVERSIONS	PERCENTAGE OF REVERSIONS
2881	101	3	2.97
2882	153	0	..
2883	95	1	1.05
2884	118	1	.85
2885	156	3	1.92
2886	118	1	.85
2887	136	12	8.82
2888	136	0	..
2889	33	3	9.09
2890	199	5	2.51
2891	115	4	3.48
2892	115	1	.87
2893	125	1	.80
2894	135	0	..
2987	103	2	1.94
2988	112	3	2.68
2989	55	5	9.09
2990	123	3	2.44
Total	2,128	48	2.26

INHERITANCE OF REDDISH-1

Experiments with reddish-1 indicated that it is an allelomorph of yellow and reddish-α; and also that, unlike reddish-α, it does not revert to the wild-type. In crosses between reddish-1 and yellow, all F_1 females were yellow; and, among 15,103 F_2 progenies, nothing but yellow and reddish flies was observed.

TIME OF OCCURRENCE OF MUTATIONS

Reversions of reddish-α to the wild-type were observed only among the progenies of females heterozygous for reddish-α. Numerous homozygous females were tested, and also several homozygous reddish-α stocks

were kept for many generations; but in no case did any change in reddish appear. Males also always behaved as constant reddish. Numerous F_1 females from crosses of yellow females by reddish males were examined, but none of them were of the wild-type. Reversions to wild-type occurring in reddish-α males would also have been noticed in stocks homozygous for reddish.

Females heterozygous for mutable reddish-α gave numerous reversions. All of these reversions affected the whole fly, indicating that the mutations occurred at the formation of the ova. If mutations occurred in somatic cells, it would be expected that mosaics would develop frequently, which was not the case. Throughout the experiments a close watch was kept for reddish and wild-type mosaics, but none was observed. If they occur at all they must be very rare. This makes negligible the hypothesis that the reversions occurred early in the somatic development.

If reversions of reddish to wild-type occurred in the oogonial divisions, one mutation would affect a group of germ cells, and the result would be that the reverted flies would appear in groups. This was not the case (table 6). In the low mutating line, usually single wild-type flies were obtained from individual females. With increase in the frequency of mutations the number of wild-type flies from single females increased also. The occurrence of reversions in groups, however, was an exception rather than a rule; which makes it improbable that the mutations occurred in the oogonial divisions, and points to the maturation division as the possible seat of the change. A relation was observed between crossing over and the occurrence of mutations, which suggests that both processes occurred at the same time, and supports the assumption that the mutations occurred at the maturation divisions.

In table 4, the data are given from the crosses between mutable reddish-α and pilose scute. From these data it can be seen that reddish mutated in the females which had in one chromosome reddish-α, and in the other chromosome the wild-type allelomorph. From the data given in tables 2 and 3, it is evident that reddish-α mutated in the females which were heterozygous for its allelomorph yellow. The data from table 8 show that reddish-α mutated in reddish-α over reddish-1 females. Reddish-α therefore mutates at the maturation divisions of females having in one chromosome mutable reddish-α, and in the other chromosome one of its allelomorphs; that is, wild-type, yellow or reddish-1. No mutation has been observed in the females homozygous for mutable reddish-α.

Wild-type which originated by a mutation from reddish-α behaves like any other wild-type; that is, it keeps the reddish-α mutating when heterozygous with it. This can be seen from the data in table 7; which

TABLE 7

The F_2 data from a cross between a reddish-α sepia female and a pilose scute vermilion male.

PEDIGREE NUMBER	♀	0		2		3		4		REVERSIONS	
		se re	*pl sc v*	*pl re*	*se sc v*	*se re sc v*	*pl*	*se re v*	*pl sc*	*sc*	*se v*
2516	47	9	13		1			5	3		
7	102	15	27			1		3	10	1	1
8	47	11	13		1			3	5	1	1
Total	196	36	53		2	1		11	18	2	2

give the F_2 results of a cross between a reddish-α sepia female and a pilose scute vermilion male. This male was a reversion obtained from a reddish-α pilose scute over sepia yellow scute vermillion female.

TABLE 8

Comparison of frequency of mutability in reddish-α/reddish-1 and reddish-α/yellow females.

PEDIGREE NUMBER	NUMBER OF MATINGS	NUMBER OF MATINGS GIVING REVERSIONS	TOTAL		PERCENTAGE OF REVERSIONS
			y and *re*	Reversions	
		Offspring of re-α/re-1 females			
2816	10	3	1160	7	0.6
2817	20	13	2506	24	0.9
2818	1	1	122	1	0.8
2819	5	2	655	5	0.8
Total	36	19	4443	37	0.8
		Offspring of re-α/y females			
2816	3	..	338	..	..
2817	29	17	3022	30	0.10
2818	1	1	93	7	7.0
2819	3	1	324	6	1.8
Total	36	19	3777	43	1.1

To determine if there was any difference in the frequency of mutations between the reddish-α over yellow and reddish-α over reddish-1 females, the same reddish-α male was mated to yellow and reddish-1 females, and the F_2 results of these crosses compared. A summary of the experiments, which were repeated four times, is given in table 8.

They indicate that reddish-α mutates with equal frequency when heterozygous with yellow or with reddish-1.

FREQUENCY OF REVERSIONS

About half of the progenies of females in which reddish-α mutates to wild-type, carry the reddish-α gene; since, as already mentioned, reddish-α can mutate in heterozygous females only. The ratio between

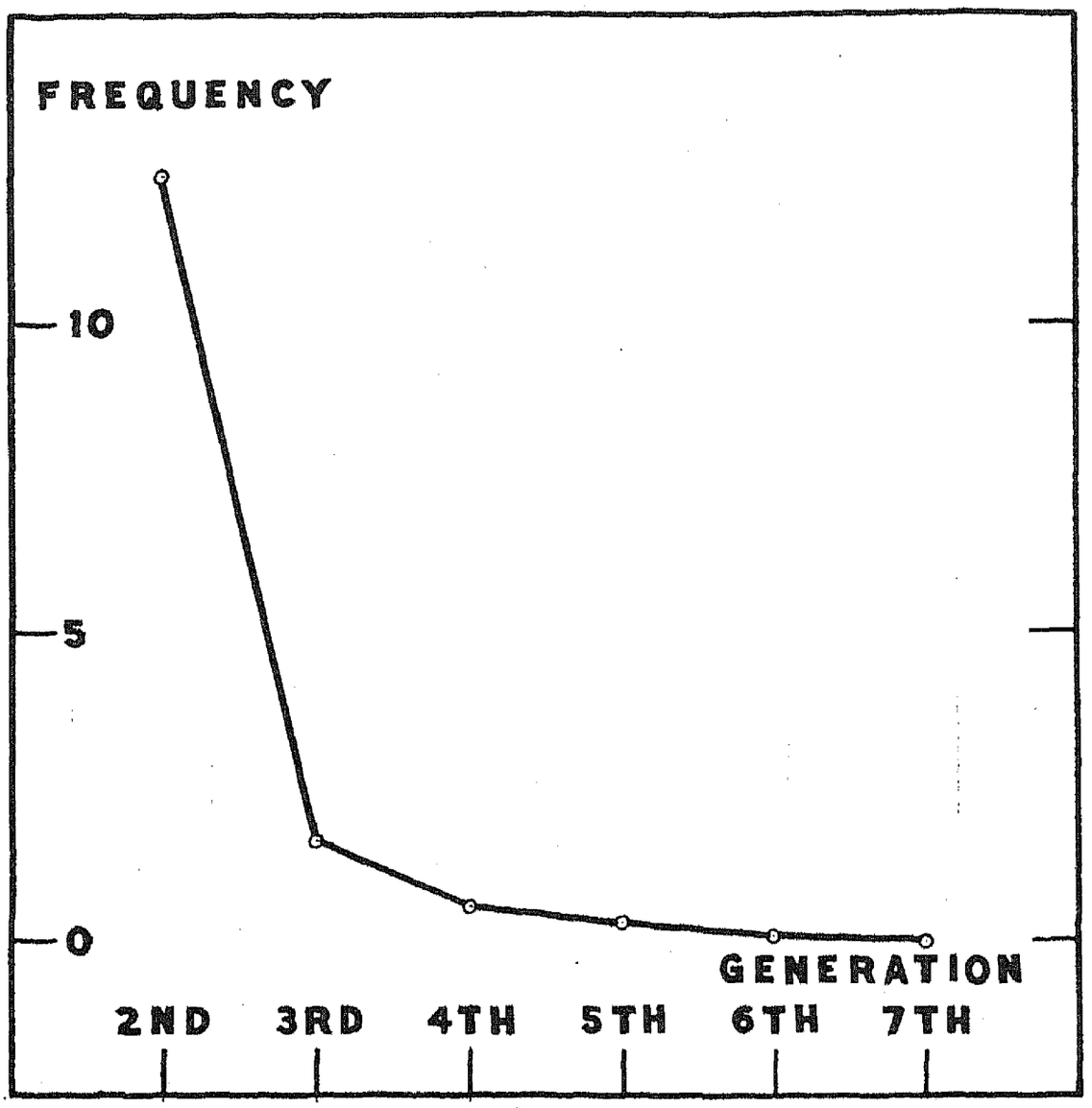


FIGURE 2.—Frequency of reversions of reddish-α to wild-type, immediately after the origin of reddish-α.

the number of reversions and the number of progenies carrying the reddish-α gene would give a measure of the frequency with which reddish-α mutated to the wild-type. Since in many experiments the reddish-α and not-reddish-α progeny had not been counted separately (and in some experiments it was not possible to separate them), the frequency

of reversions throughout this paper is expressed not by the proportion between the number of reversions and the number of progeny carrying the reddish-α gene, but by the proportion between the number of reversions and the total number of the progeny. Since approximately only half of the total number of progeny of heterozygous females was expected to carry the reddish-α gene, the "frequency" of reversions as the term is used in this paper will amount to about half of the actual frequency.

Mutability of reddish-alpha at the time of origin

The progenies of yellow reddish-α females offer the best material for the determination of the frequency with which reddish-α reverts to wild-type; since among these progenies all classes, namely, yellow,

TABLE 9

Frequency of reversions of reddish-α to wild-type, immediately after the origin of reddish-α.

GENERATION	TOTAL NUMBER OF FLIES	NUMBER OF REVERSIONS	FREQUENCY OF REVERSIONS PERCENTAGES*
2nd	241	30	12.4
3rd	9633	152	1.6
4th	8022	48	0.6
5th	2147	7	0.32
6th	11532	5	0.043
7th	4041	0	0.0

* Since the reddish gene was present in half of the gametes only and in calculations total number of flies was used, the frequency of reversions was about twice as large as given in this column.

reddish-α and reversions, can easily be distinguished. When reddish-α was found, one of the first crosses made with it was with yellow. The same cross was repeated for different purposes through many generations. This made it possible to determine the frequency with which reddish-α reverted in different stages of the experiments.

Fig. 2 represents the curve and table 9 gives the actual data on the frequency with which reddish-α mutated to the wild-type during the second to seventh generation after it was found. At that time the matings were made at random, without any selection to increase the frequency of of reversions. As can be seen from both fig. 2 and table 9 the frequency reversions decreased rapidly in succeeding generations; until in the seventh generation no reversion was found among 4041 individuals. In the second generation after the origin of reddish-α, the females heterozygous for reddish-α gave 12.4 percent of reversions; two gener-

ations later, similar females gave 0.6 percent of reversions; and in the sixth generation, among 11,532 offspring, only 5 reversions were observed.

Effect of selection on the mutability of reddish-α

The mutable reddish-α stock was kept up for a long time by back-crossing reddish-α over sepia yellow scute females with sepia yellow scute males. Since there is no double crossing over between sepia, yellow and scute, all yellow females from that cross had the genetic constitution of their mother. It was therefore possible to perpetuate the cross by mating yellow females with sepia yellow scute males from the same culture.

TABLE 10

Effect of selection on the frequency of reversions.

GENERATION	TOTAL NUMBER OF FLIES	NUMBER OF REVERSIONS	PERCENT OF REVERSIONS
1	591	23	3.9
2	196	7	3.6
3	578	6	1.0
4	45	4	8.9.
5	180	8	4.4
6	92	2	2.2
7	415	14	3.4
8	491	12	2.4
9	171	3	1.8
10	362	4	1.1
11	795	21	2.6
12	796	25	3.1
13	5731	145	2.5
14	7625	99	1.3
15	5727	276	4.8
16	4890	178	3.6
Total	28,685	827	Average 2.9

Pair matings were always used to propagate a line. Since reddish-α mutates in heterozygous females only, this was the simplest way to keep a reddish-α stock which was certain to be mutating.

If many pair matings were made from a culture which gave a high number of reversions, the number of reversions in the offspring varied from zero to as high as, or higher than, in the parent culture. It has been shown before that the frequency of reversions decreased rapidly if the matings were made at random. If, however, the matings were made from cultures which gave a high number of reversions, the frequency of reversions did not decrease, but remained more or less constant. That can be seen from table 10, which records the results of an experiment

where selection was carried on for sixteen generations with the purpose of increasing the frequency with which reddish-α mutated. During that time the line was propagated from the cultures which gave the highest number of reversions. By this selection increase in the mutability was not attained, but neither did a decrease occur. The mutability in the line remained approximately the same. In individual cultures, the number of reversions was as high as 25 percent; but in the totals of different experiments the mutability rarely exceeded 10 percent.

A relation was found to exist between the mutability of the parent culture and the mutability of the offspring. Offspring of a highly mutable culture are likely to give more highly mutable cultures than the offspring of a less mutable one (table 11). The increase in the mutability, however, does not go behind a certain limit which is between three and five percent.

TABLE 11

Comparison between the mutability of parents and offspring.

MUTABILITY OF PARENT CULTURE PERCENT	NUMBER OF CULTURES	DISTRIBUTION OF MUTABILITY IN OFFSPRING										TOTAL
		0		1 Percent		1-5 Percent		5-10 Percent		10 Percent		
		Number of cultures	Percent	Number of cultures	Percent	Number of cultures	Percent	Number of cultures	Percent	No. of cultures	Percent	No. of cultures
0	17	88	77.9	5	4.4	13	11.5	5	4.4	2	1.8	113
less than 1	16	72	64.3	15	13.4	17	15.2	7	6.3	1	0.8	112
1–5	32	65	37.6	11	6.4	62	35.8	29	16.8	6	3.4	173
5–10	19	10	11.8	7	8.2	39	45.9	22	25.9	7	8.2	85
more than 10	7	5	25.0	1	5.0	9	45.0	3	15.0	2	10.0	20

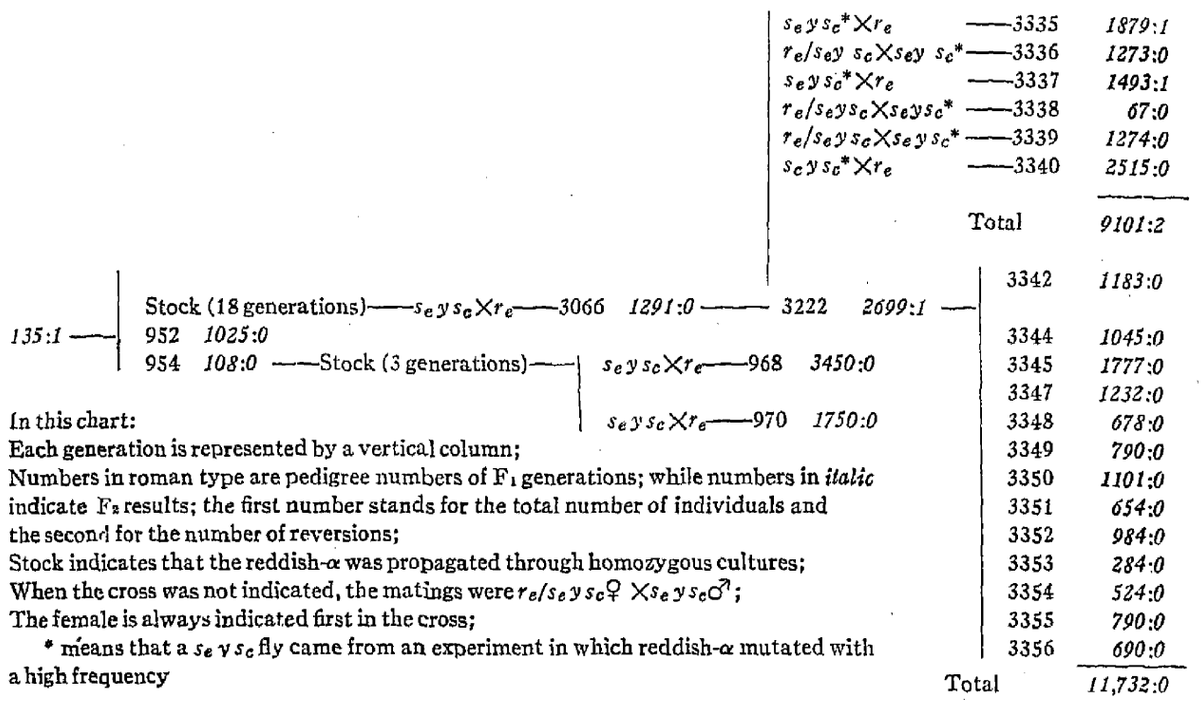
Constancy of reddish-alpha

As can be seen from table 11 constant cultures (with no reversions) were found among the offspring of highly as well as among the offspring of little mutable cultures. This was almost without exception. The lack of reversions was probably due in some of the cultures to the small numbers of individuals; in others, however, it was probably caused by the constancy of reddish-α.

Two males were taken from a very mutable culture which gave 16 reversions out of 97 individuals. They were mated to sepia yellow scute females from stock. In the F_2 generation from one of the matings, among 1307 flies, there was only one reversion; and in the F_2 from the other mating there were no reversions among 425 flies. Both of the males

came from a highly mutable culture; but offspring of one of them had very low mutability, and the offspring of the other was either of very low mutability or possibly constant. The number of individuals in this experiment was large enough to indicate a difference in mutability between the parents and the offspring.

It is evident from the chart that reddish-α can become constant or nearly so. Three lines originated from a culture which had very low mutability and which gave one reversion among 135 individuals. One of these lines was discontinued after one F_2 generation had been obtained in which no reversion was found among 1025 individuals. The other line



was continued for several generations, and 5308 offspring of females heterozygous for reddish-α were examined, none of them being a reversion. In these two lines reddish-α behaved as a constant gene in a total of 6333 individuals. The third line was kept as homozygous reddish-α for about eighteen generations. To test its mutability it was crossed to sepia yellow scute; and further propagated by backcrossing with sepia yellow scute males, and through crosses between reddish and sepia yellow scute flies. Among 24,823 flies of this line there were only three reversions. It might be questioned, however, whether these three reversions did not originate in a different way to the others, since all three were crossovers in the yellow-scute region. It is unlikely that they came into the experiment by contamination, since at that time no stock of the same genetic constitution was in existence in the laboratory. In any case, the frequency of

TABLE 12

Comparison of the frequency of reversions among the offspring of young and old females.

PEDIGREE NUMBER	NUMBER OF MATINGS	♀'s			♂'s										TOTAL ♀ AND ♂		
		Total	Reversions		0		1		2		Reversions		Total				
		r_e and y	+	s_c	r_e	$s_e y s_c$	$s_e r_e$	$y s_c$	$r_e s_c$	$s_e y$	+	s_c	r_e and y	Reversions	r_e and y	Reversions	Percentage of reversions
Offspring of young females																	
1283	10	453	21	4	205	182	2	6	2	2	22	3	399	25	852	50	5.9
1232	5	131	5	1	65	59	1	..	..	2	1	..	127	1	258	7	2.7
1285	1	9	1	..	13	1	..	..	..	..	1	..	14	1	23	2	8.7
1296	2	222	1	2	86	86	..	1	1	2	4	1	176	5	398	8	2.0
1299	2	84	5	2	47	48	..	..	..	..	3	1	95	4	179	11	6.1
1300	1	86	1	..	41	42	1	1	..	1	..	..	86	..	172	1	.6
1301	1	55	3	1	27	41	..	1	..	..	3	..	69	3	124	7	5.6
1547	2	138	5	..	43	62	4	1	1	1	6	1	112	7	250	12	4.8
1684	2	92	..	..	53	33	1	..	..	..	1	..	87	1	179	1	.6
1664	1	64	..	..	18	17	1	1	..	..	1	1	37	2	101	2	2.0
1727	1	19	1	1	12	9	..	..	..	..	2	1	21	3	40	5	12.5
1728	4	212	4	..	96	107	4	..	1	1	1	1	209	2	421	6	1.4
1730	1	82	2	..	37	38	..	1	..	..	1	..	76	1	158	3	1.9
1731	2	63	2	1	51	45	..	..	..	..	3	1	96	4	159	7	4.4
1732	5	268	7	1	97	139	..	2	3	..	4	..	236	4	504	12	2.4
1738	1	69	..	1	27	22	1	..	..	..	1	..	50	1	119	2	1.7
1607	1	75	1	..	39	43	1	1	..	..	1	..	84	1	159	2	1.3
1646	7	420	11	1	152	156	6	2	..	2	7	2	318	9	738	21	2.8
Total	49	2542	70	15	1109	1125	22	17	8	11	62	12	2292	74	4834	159	3.3

TABLE 12 (Continued)

Comparison of the frequency of reversions among the offspring of young and old females.

PEDIGREE NUMBER	NUMBER OF MATINGS	♀'s			♂'s										TOTAL ♀ AND ♂		
		Total	Reversions		0		1		2		Reversions		Total				
		r_e and y	+	s_e	r_e	$s_e y s_e$	$s_e r_e$	$y s_e$	$r_e s_e$	$s_e y$	+	s_e	r_e and y	Reversions	r_e and y	Reversions	Percentage of reversions
Offspring of old females																	
1283	10	468	2	..	198	212	8	4	1	1	3	..	424	3	892	5	.6
1232	5	268	..	..	109	79	3	2	..	..	..	..	193	..	461	..	..
1285	1	63	..	..	25	12	1	..	..	..	..	..	38	2	101	2	2.0
1296	2	113	1	..	45	50	..	1	..	..	1	1	96	2	209	3	1.4
1299	2	156	3	..	71	67	..	2	..	..	2	..	140	..	296	3	1.0
1300	1	62	..	..	25	21	1	1	..	..	..	..	48	..	110	..	..
1301	1	51	3	1	21	24	1	..	..	..	1	1	46	2	97	6	6.2
1547	2	114	3	..	53	50	1	1	..	2	4	..	107	4	221	7	3.2
1684	2	136	..	..	44	41	2	2	..	..	..	..	89	..	225	..	..
1664	1	50	..	..	14	19	1	..	..	..	1	..	34	1	84	1	1.2
1727	1	50	1	..	20	26	..	..	..	..	..	..	46	..	96	1	1.0
1728	4	166	..	..	89	65	1	1	1	2	1	..	159	1	325	1	.3
1730	1	48	1	..	24	24	..	..	..	..	..	..	48	..	96	1	1.0
1731	2	75	2	..	30	25	..	..	1	..	3	..	56	3	131	5	3.8
1732	5	256	3	..	123	125	..	1	4	..	1	1	253	2	509	5	.98
1738	1	17	..	..	10	8	..	..	..	..	..	..	18	..	35	..	..
1607	1	50	..	..	20	16	1	..	1	..	..	..	38	..	88	..	..
1646	7	322	2	1	145	159	2	2	2	4	9	1	314	10	636	13	2.0
Total	49	2465	21	2	1066	1023	22	17	10	9	26	4	2147	30	4612	53	1.1

reversions was so low as to be almost negligible; and reddish-α of that line behaved more like a constant than like a mutable character.

The evidence accumulated from the experiments with mutable miniature-α character of *Drosophila virilis* indicated that the mutablity of the miniature-α gene was affected by several other genes. It has been found that, in the presence of a certain dominant autosomal gene, an almost constant miniature-α becomes highly mutable. To determine if the absence of the proper modifying factor was responsible for the constancy of reddish-α, six crosses were made between reddish-α from the constant line and sepia yellow scute flies from the experiments in which reddish-α mutated with a high frequency. These crosses are marked with an asterisk on the chart. From the results it can be seen that the mutability of reddish -α was not influenced to any appreciable extent. Among a total of 9101 flies, only two reversions were obtained (both of which were crossovers in the yellow-scute region). This makes it probable that the constancy of reddish-α was due to the gene itself rather than to the absence of factors which would increase the mutability.

EFFECT OF THE AGE OF THE FEMALE ON THE FREQUENCY OF MUTATIONS

In the course of experiments with mutable reddish-α, it has been noticed that the proportion of reversions was higher among the first hatched flies than among the flies which hatched later. This difference in the proportion of reversions among the early and late hatched offspring could be accounted for in two ways. Either the reversions hatched sooner than the other classes, or the proportion of reversions was larger in the first laid eggs. To determine which of these two possibilities was more probable, a comparison was made between the offspring which came from the eggs laid by young females, and the offspring from the eggs laid by the same females when old. In several experiments, pairs were kept in one set of culture bottles until the first offspring began to hatch, and then they were transferred to new culture bottles. Offspring in the first bottles came from the eggs laid by the females younger than four weeks, and the offspring in the second bottles came from females older than four weeks. The summary of the data of eighteen experiments involving 49 females is given in table 12, from which it can be seen that, in all except one experiment, the percentage of reversion was larger among the offspring of young females. When the data of all the experiments are added, the percentage of reversions in the offspring of young females is three times the percentage of reversion in the offspring of old females.

The result of this experiment indicates that the age of the flies influences the frequency of mutations of reddish-α to wild-type. In old females, that frequency is appreciably lower than in the young females.

CROSSING OVER AND REVERSIONS

As has been already pointed out in one of the previous chapters, crossing over can not be responsible for the appearance of reversions in crosses with reddish-α. The behavior of reddish can not be compared with the behavior of bar in *Drosophila melanogaster*; where, as has been shown by STURTEVANT (1925), the appearance of unexpected wild-type flies was always connected with crossing over in the bar region. In the case of reddish-α (tables 2, 3, 12 and 13) about 87 percent of reversions are non-crossovers in the regions adjoining reddish.

There is, however, a connection between crossing over and reversions of reddish-α. An examination of the data given in table 13 reveals a marked increase of yellow-scute crossovers in reversion classes as compared with the same crossovers of normal classes. The values given in table 13 are directly comparable, since all the data were obtained from

TABLE 13

Male offspring of s_e y s_c v/r_e females showing the amount of crossing over in different regions.

0	r_e $s_e\, y\, s_c\, v$	7589 7230
1	$r_e\, s_c$ $y\, s_c\, v$	195 146
2	$r_e\, s_c\, v$ $s_e\, y$	50 88
3	$r_e\, v$ $s_e\, y\, s_c$	1790 1785
1–3	$s_e\, r_e\, v$ $y\, s_c$	.. 5
2–3	$r_e\, s_c$ $s_e\, y\, v$	2 3
Reversions	+ v $s_c\, v$	255 48 46
Total		19,232

the same females. Unequal distribution of crossovers is still more evident from the analysis given in table 14, where the data for reddish and reversion classes of table 13 are brought together. From table 14 it can be seen

TABLE 14

Comparison between crossovers occurring in reddish and reversion classes.

CROSSOVER REGIONS	CLASSES		PERCENT OF REVERSIONS
	Reddish	Reversions	
0	7589	255	3.3
$s_e - r_e$	195	0	0.0
$r_e - s_c$	52	46	46.9
$r_e - v$	1790	48	2.6
Totals	9626	349	3.5

that among the crossovers in reddish-scute region in 46.9 percent of possible cases reddish-α reverted to the wild type. On the other hand, in non-crossover class reddish-α reverted in 3.3 percent of possible cases, in the sepia-yellow crossover class there were no reversions and in the scute-vermilion crossover class the reddish-α reverted in 2.6 percent of possible cases.

From table 14 it could be seen also, that among 9626 reddish-α flies there were only 52 (0.54 percent) crossovers in reddish-scute region, while among 349 reversions there were 46 (13.2 percent)crossovers in the same region. The percentage of yellow-scute crossovers in reddish classes is lower than it would be normally expected. That deficiency, however, was probably caused by a poor viability of scute flies. An examination of the data given in table 13 shows that the scute class was in all except one case lower than its reciprocal class. A comparison of crossover percentages reveals that the crossing over in reddish-scute region was 24.4 times as frequent among reversions as among the normal classes.

The data presented above indicate that almost one half of the crossovers in reddish-scute region were reversions, or, when examined from a different angle, they show that the crossing over in reddish-scute region was 24.4 times as high among the reversions as among the normal classes. The observed facts may be accounted for in two ways: (a) by the assumption that the total amount of crossing over in reddish-scute region was not influenced by the occurrence of reversions but that the reversions occur about twelve times as frequently when the crossing over in reddish-scute region occurs than when there is no crossing over, or (b) by the assumption that whenever the reversion occurs in one out of about thirteen cases

crossing over in reddish-scute region will occur also, irrespective of whether this crossing over would have occured otherwise. According to the first assumption, it would be expected that the increase in the frequency of reversions would not change the total amount of crossovers in the reddish-scute region, but would only affect their distribution between the reversions and the normal classes. According to the second assumption, an increase in the amount of reversions would also result in the increase in the total of reddish-scute crossovers. According to either of the assumptions, however, the occurrence of reversions is stimulated with the close association of homologous chromosomes in reddish-scute region at the time when crossing over occurs.

In table 15 the data are summarized according to the amount of rever-

TABLE 15

Comparison between the frequency of reversions and the percentage of crossing over in the r_e-s_c region

FREQUENCY OF REVERSIONS IN DIFFERENT EXPERIMENTS. PERCENT	AVERAGE FREQUENCY PERCENT	TOTAL NUMBER OF FLIES	PERCENT OF CROSSOVERS IN r_e-s_c REGION		
			Reversions	r_e and y classes	r_e and y and reversions
less than 0.5	0.3	4594	13.3	0.6	0.7
.5–1.0	0.8	3109	15.4	0.7	0.9
1–2	1.6	1348	9.5	0.5	0.6
2–3	2.3	2436	10.7	1.1	1.3
3–4	3.2	1455	6.5	1.1	1.2
more than 4	5.2	1625	16.5	1.1	1.8

sions obtained in different experiments. The results indicate that the amount of crossing over among reversions was approximately the same in the experiments which had a low frequency of reversions as in those in which the frequency was high. On the other hand, there is an indication that in the experiments which had a high frequency of reversions there was an increase in the total crossing over as well as in the percentage of crossing over in the reddish and yellow classes. These findings favor the second assumption.

It is unfortunate that it has not been possible to increase the amount of reversions much above five percent. The low frequency of reversions makes the reversion classes small and so prevents a more detailed analysis of the relation between the occurrence of reversions and crossing over in the reddish-scute region.

DISCUSSION

From the evidence obtained in different experiments with reddish-α the most fitting explanation for the unusual behavior of that character

seems to be the hypothesis that the gene for reddish-α reverts frequently to the gene for the wild-type. Several other hypotheses, which could explain the origin of wild-type flies in crosses with reddish-α, fail entirely to stand the crucial tests.

Any hypothesis explaining the behavior of reddish-α would have to assume that reddish-α is a sex-linked character and also an allelomorph of yellow. There can not be any question as to the validity of the first assumption. In the numerous matings in which reddish was used it never failed to show the characteristic behavior of a sex-linked character of Drosophila. The assumption of allelomorphism to yellow is well supported by: (a) linkage tests, which indicate that reddish-α is located in approximately the same region as yellow; (b) F_1 female progenies from crosses between reddish-α and yellow, which are always yellow; (c) F_2 results from these crosses, where only reddish-α and yellow males were obtained if the reddish-α parent used in the cross was from a constant line; and (d) by the fact that the reddish-α and yellow affect the same character, as it is the case with all known allelomorphs.

When reddish-α of a "mutable" line is crossed with yellow, all F_1 females are yellow, as expected; but among F_2 progenies, in addition to yellow and reddish-α flies, a few wild-type individuals appear. The number of wild-type flies varies in different cultures from zero to 20 percent. It is usually low, and rarely reaches above 10 percent. What is the origin of these unexpected wild-type flies? In addition to the hypothesis that they are due to the change of the gene for reddish-α to the gene for wild-type, is possible that they might be a product of: (a) the variablity of reddish-α; (b) recombination between reddish-α and yellow; (c) the complex nature of the reddish-α character; or (d) the abnormal behavior of chromosomes. Let us consider these possibilities in detail.

(a) If the exceptional wild-type flies were due to the variability of reddish-α, it would be expected that they would be genetically reddish-α. That, however, was not the case. A number of these flies was tested for two generations and they gave only wild-type progenies, without any reddish-α flies.

(b) It has been shown by STURTEVANT (1925) that in exceptional cases unequal crossing over occurs in the bar-region of sex chromosome of *Drosophila melanogaster*, and results in the recombination of allelomorphs. Similar process occuring in the reddish-α region could produce the wild-type flies. In that case, however, it would be expected that all wild-type flies would be crossovers. Quite the opposite was found, since only about

13 percent of the exceptions were yellow-scute crossovers, and almost none were crossovers in the sepia-yellow region. These results eliminate the possibility of the wild-type flies originating by unequal crossing over.

(c) By assuming that the reddish-α character is determined by duplicate genes a scheme could be devised which would explain the appearance of wild-type flies. The abnormally small and variable numbers of the wild-type flies could probably be explained by the assumption of differential viability. There are, however, other and more serious obstacles to that hypothesis. If the reddish-α character were determined by duplicate genes, one of them would be undoubtedly sex-linked and an allelomorph of yellow, and the other or others might be either autosomal or sex-linked. If any of the genes needed for the expression of the reddish-α character were an autosomal recessive, reddish-α would not behave as a sex-linked character. If it is assumed, however, that the autosomal gene is a dominant one, it would be expected to segregate freely with the gene in the sex chromosome. The expected result would be that the wild-type flies would appear among the progeny of heterozygous males as well as among the progeny of heterozygous females. That was not the case. Males from the cross reddish-α by yellow produced no wild-type flies, while the females from the same cross gave wild-type flies.

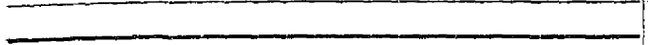
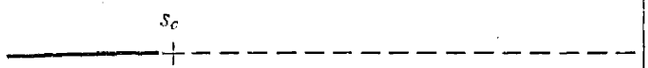
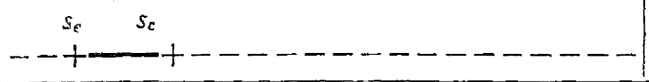
There are several reasons which eliminate the possibility of reddish-α being determined by duplicate genes located in the sex chromosome. It might be enough to consider one of them. If it is assumed that reddish-α is determined by two sex-linked genes, one of which is an allelomorph of yellow, then the wild-type flies observed in crosses between reddish-α and yellow, would constitute half of the crossovers between the duplicate genes determining reddish-α. It has been shown in table 14 that, in an experiment carried through sixteen generations, the average frequency of wild-type flies was about three percent. That would indicate that the other duplicate gene of reddish-α would have to be located about six units from yellow. If the wild-type flies were crossovers between the two duplicate genes it would be expected that all of them would be crossovers in the region six units on either side of yellow. The data given in table 10 show that they are not crossovers in the yellow-vermilion region, and also indicate that it is very unlikely that they are crossovers in the region on the other side of yellow.

(d) In all experiments, reddish-α showed a strictly sex-linked inheritance that eliminates the possibility of the unexpected behavior of reddish-α being due to an abnormal action of the autosomes. In numerous experiments made with reddish-α there was nothing to support the as-

sumption that the abnormal behavior of a whole sex-chromosome was responsible for the origin of the wild-type flies obtained in crosses with reddish-α. In table 16, the data are given which show that the wild-type

TABLE 16

Summary of experiments in which different regions of the original reddish-α chromosome were represented. (Solid line indicates the left-end of the original reddish-α chromosome and the broken line the replaced regions.)

TYPE OF THE X-CHROMOSOME	NUMBER OF EXPERIMENTS	NUMBER OF FLIES	
		Total	Reversions
	66	37,462	684
	12	4,549	84
	2	1,459	19
	33	15,686	223
	11	5,580	51
	9	4,214	35

flies occured in the experiments in which the whole original chromosome carrying reddish-α was present as well as in the crosses in which different parts of that chromosome were replaced by the parts from the other sex chromosome. From table 16 it could be seen that the small part of reddish-α chromosome located between sepia and scute (about 3 units in length) was sufficient to produce the abnormality; and the data discussed on page 384 indicate that a gene, rather than a region of the chromosome, is responsible for the origin of the wild-type flies.

Reddish-α was found to mutate in one direction only, namely, from reddish-α to wild-type. Tests involving a large number of progenies of reverted reddish-α failed to detect any mutation in the opposite direction. Moreover, the mutability of reddish-α was found to be limited to a very short period of development, which has been located at the maturation divisions of heterozygous females. No mutation was observed in somatic cells, in homozygous females, or in males. What could be the reason for that narrowly restricted mutability of the reddish-α gene? Experimental evidence does not give as yet any answer to this question. It may be possible that the restriction is due to the quality of the gene itself, or it may be also possible that the reddish-α gene is potentially mutable in other periods of development, but that the stimulus which produces the

immediate change is lacking except at the maturation divisions of heterozygous females. It has been found that the potentially mutable gene of miniature-α behaves almost as a constant one until it is stimulated to mutate by some other gene. Two such genes were isolated; both autosomal dominants. One of them induces miniature-α to mutate with high frequency in somatic cells, but does not affect the mutability of germ-cells. The other one induces a high mutability of the germ-cells, not changing appreciably the mutability of somatic cells. Some condition similar to that found in the case of miniature-α would account for the restricted mutability of reddish-α. The attempt, however, to induce somatic mutability in reddish-α by the gene which stimulates miniature-α to mutate somatically, was not successful.

Restriction of the mutability of a mutable gene to limited periods in the development of the organism is not an unusual condition. IKENO (1923) described two recessive characters in Plantago, which when selfed, gave regularly a small number of the wild-type individuals. These wild-type plants were interpreted as due to reversions occurring at the formation of gametes, or in the somatic cells closely preceding the reduction division. Mosaic plants, which would be due to mutations during the late somatic development, were observed very rarely, if at all. Similarly IMAI (1925a) found that the cream-colored flower character of Pharbitis when selfed gave regularly about 6 percent dark colored plants. Variegated flowers were rarely observed. In a line of Antirrhinum, BAUR (1926) obtained regularly about 2 percent crispa plants. Crispa is a dominant character and lethal when homozygous. The origin of crispa plants could be interpreted as being due to the mutability of the gene for the wild-type to the gene for crispa. Since no mosaic plants were observed the mutability in this case was probably limited to maturation divisions. IMAI (1925b) described a mutable character in Pharbitis which had the gametic frequency of mutations of 0.40 percent, and the somatic frequency of 0.17 percent. In Delphinium (DEMEREC 1927b) a character is known which mutates with high frequency in the early stages of the development of plants, and with very high frequency in the late stages of development, but does not mutate at all or mutates with a very low frequency in the intermediate stages. The difference between the mutability of reddish-α and the mutability of the genes just mentioned appears to be only in the fact that the period of the mutability of reddish-α is still more limited than the period of mutability of these genes. Consequently the difference between the mutability of reddish-α and such genes producing variegation in plants would be that these genes mutate at all stages of the develop-

ment, while reddish-α mutates at the maturation divisions of heterozygous females only.

An attempt has been made (CHITTENDEN 1927) to explain the behavior of variegated pericarp of maize and other similar variegations found in plants as due to the plastid inheritance. The reddish-α case, however, is distinctly chromosomal in inheritance and any hypothesis to explain the observed behavior as being determined by cytoplasm seems to be inconceivable.

SUMMARY

Reddish-α was found in two sister matings in the backcross involving the character branched as well as concave approximated and telescoped. In one of the matings, about one half of the males were reddish; and in the other only one reddish male was found. The possibility is not excluded, but the probability is low, that the single male came as a contamination from the culture which had many reddish males.

Reddish-1 was found (by Miss M. MOSES) seventeen months later in a mating involving the same characters as the one in which reddish-α was found. The branched used in the second case had a different origin from the branched used in the first case, but the concave approximated telescoped flies came from the same line.

To test the possibility that in the concave approximated telescoped line mutations to reddish occur frequently; 16,980 offspring of the backcrosses involving branched, approximated and telescoped characters were examined. No reddish flies were found.

Both reddish-α and reddish-1 are sex-linked and allelomorphs of yellow.

In F_2 generations from crosses between reddish-α and yellow, in addition to reddish-α and yellow flies, few wild-type ones appear. Experiments indicate that these wild-type flies are not due to crossing over between reddish-α and yellow, that they are not due to the abnormal behavior of chromosomes, nor to the complex genetic nature of reddish-α. They are interpreted as being reversions of the gene for reddish-α to the gene for wild-type.

The wild-type flies which originated as reversions from reddish-α are genetically constant.

Reddish-1 does not revert to wild-type.

Reddish-α reverts to wild-type only in females which are heterozygous for reddish-α and one of its allelomorphs, namely, wild-type, yellow or reddish-1. No reversions were observed in somatic-cells, in homozygous females, or in the males.

The frequency of reversions was found to be very variable. It decreased from 12.4 percent in the second generation after the origin of the reddish-α, to zero in the seventh generation.

By using for matings flies from cultures which gave the highest number of reversions it has been possible to keep the frequency of reversions for sixteen generations at about three percent.

A positive relation was found to exist between the mutability of the parent culture and the mutability of the offspring.

A line was isolated in which reddish-α behaved as an almost constant character.

The age of the female reduces the frequency of reversions.

It was found that, in the classes which originated by reversions, the crossing over in the yellow-scute region was increased 24 times over the normal amount. An analysis of the data indicates that the force which influences the reddish-α to revert to the wild-type at the same time causes the increase in the crossing over in the yellow-scute region.

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TWO NEW MUTANT CHARACTERS ON THE SPERMATHECAE OF THE FEMALES OF *DROSOPHILA MELANOGASTER*. CELL-DEGENERATION AND SUPERNUMERARY SPERMATHECAE.

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INTRODUCTION

In this work two mutant characters have been studied, both affecting the normal development of the spermathecae in the females of *Drosophila melanogaster*. The first character is a hereditary degeneration of the cells of the epithelium of the spermathecae. It was found in January 1921 by Doctor J. L. COLLINS as a mutation in a stock of curved flies which was extracted from a cross, black purple curved×jaunty. The second is the occurrence of the supernumerary spermathecae, a stock being produced in which the females all showed 3 instead of the normal number 2 spermathecae. This mutant was found in the stock which carried the hereditary cell-degeneration. The present investigation was carried out in the Genetics Laboratory of the UNIVERSITY OF CALIFORNIA during the fall semester of 1926 and the spring semester of 1927. I take great pleasure in thanking Professor E. B. BABCOCK for the facilities given me in the Genetics Laboratory and Doctor J. L. COLLINS for the material and for help and suggestions during the course of the work. Acknowledgment is also given to the INTERNATIONAL EDUCATION BOARD for the fellowship granted to me.

THE HEREDITARY CELL-DEGENERATION

Description

As far as it has been possible to observe the cell-degeneration is strictly limited to the epithelium of the spermathecae. The gross appearance is

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1, 2 or 3 black spots on the dorsal side of the posterior part of the abdomen (figure 1). When highly developed it can be seen with the naked eye; if the degeneration is slight, however, the spermathecae are hard to see, even with large magnification and a dissection of the fly is often necessary

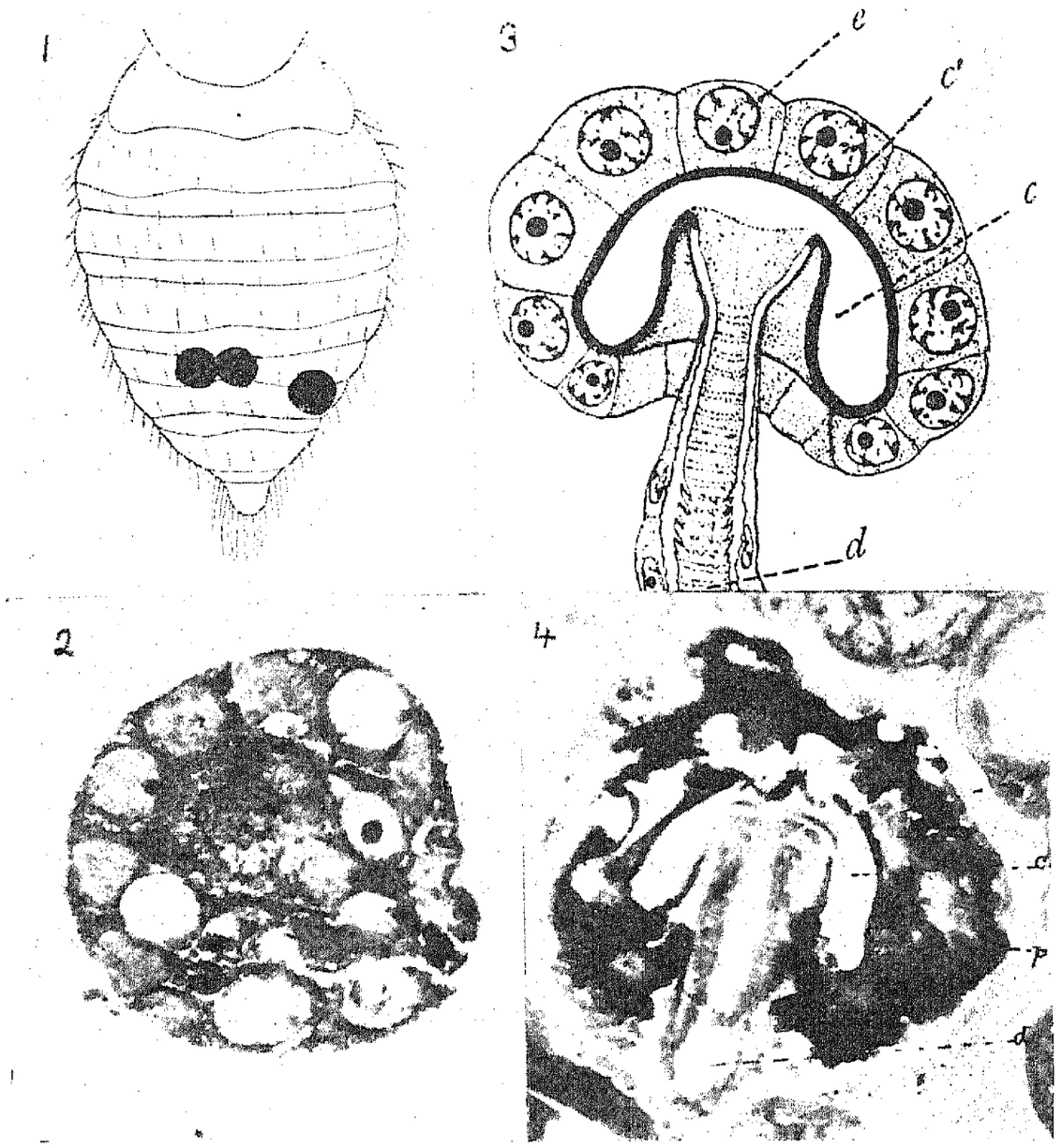


FIGURE 1.—Abdomen of female carrying 3 spermathecae each with a cover of degeneration tissue.

FIGURE 2.—Cross-section of the epithelial layer of a spermatheca at an early stage of degeneration. No nuclei are found in the epithelial cells which are filled with a brown pigment. Microphotograph Magnification ×800 diam. 5μ. Haematoxylin.

FIGURE 3.—Longitudinal section of a normal spermatheca; c=cavity; c^1=cuticle; d=duct; e=epithelium. Photograph after drawing of NONIDEZ (1920, p. 215).

FIGURE 4.—Longitudinal section of a highly degenerated spermatheca. Brown pigment covers most of the epithelium in which all cellular structure is lost. Microphotograph Magnification ×800 diam. 5μ. Haematoxylin. c=cavity; d=duct; e=epithelium; p=pigment.

to make a safe decision. The spermathecae are 2 (or 3) mushroom-shaped bodies connected with the uterus by narrow ducts and serving as storage organs for the sperm. Their terminal cavity is surrounded by a brown cuticle, secreted by the epithelium forming the walls of the organ (figure 3).

As the degeneration proceeds there is a deposition of dark brown pigment in the epithelial cells. Figure 2 shows a cross-section of the epithelial layer at an early stage of pigment formation. The cell structure is still retained, but the nuclei are gone and the cells are filled with a brown pigment. Later on the cell structure is completely gone and the brown pigment forms a complete bag around the spermathecae. A section through such a spermatheca is shown in figure 4.

The deposition of pigment seems often to be checked at an early stage and the gross appearance is then a few brown granules scattered throughout the epithelial layer, a type which I have called d_g-*a* for sake of convenience as contrasted with d_g-*b* in which the pigment forms a complete bag around the spermathecae, and is visible as conspicuous black spots such as shown in figure 1. There seem to have been present in the stock modifiers, reducing the pigment deposition, for by selection, the percentage of d_g-*b* females was considerably raised.

However, even after 15 generations of selective inbreeding both types occur, showing that they may appear as modifications of the same genotype. The males of the d_g stock are normal as far as has been observed, but no dissection of males has been made. The data given below on development and inheritance are therefore concerned with the females only.

Development and variability

The degeneration has never been observed in larvae or pupae and indeed not in newly hatched flies. Observations were made on 42 virgin females which were isolated at the pupal stage (throwing out the males as soon as possible after emergence). The females were examined as often as possible during the first 72 hours; visible pigment did not develop until 24 hours after emergence and developed in some cases as late as 72 hours after emergence. Classification of d_g *versus* non-d_g flies can therefore be safely made only on females at least 3 days old; a circumstance which complicates the genetic analysis considerably. By counting the flies twice a day, for instance, one could run pure cultures of the mutant stock, finding only a single individual with degenerated spermathecae, now and then. If only sufficiently-old females are examined, the degeneration frequency in pure cultures is now 100 percent.

There is still variation in the degree of degeneration and a few experiments were carried out to determine the influence of external factors. Breeding at 24° and 31° C did not give any difference in the degree of degeneration, nor did a variation in the moisture of the cultures have any effect. To test the effect of the amount of food, vials were made up with 5cc banana-agar food in each and in these were placed 50, 100, 150 or 200 eggs from cultures of the mutant stock. The results are given in table 1 under 2 groups (1) 50-100 eggs—(2) 150-200 eggs. The degree of cell-degeneration is expressed by the relative proportion of d_g-a and d_g-b flies.

TABLE 1

Effect of amount of food upon cell-degeneration.

NUMBER OF BOTTLES	NUMBER OF EGGS PER BOTTLE	AVERAGE NUMBER OF FLIES	d_g-a	d_g-b
12	50–100	18.42	44	52
6	150–200	42.80	38	105

Although the numbers are small, there is a clear indication of increasing degeneration with decreasing amount of food.

It is not likely that the degeneration is due to infection or some other external cause as it segregates out in Mendelian ratios. The sex-ratio in the mutant stock is normal, which shows that the females with the abnormal spermathecae are equal in viability to the males which are normal. The viability of the d_g classes in relation to the non-d_g classes in segregating populations is discussed under the treatment of the results from the backcrosses.

The fertility of the females is good. It has not been possible to ascertain whether the sperm can enter an abnormal spermatheca, and whether such a spermatheca can again give off sperm for fertilization, but this seems likely in view of the good fertility of the females. It was thought that fertilization was perhaps necessary for the cell-degeneration, as the development does not begin till after the time when the females have usually been fertilized in mixed cultures. The foregoing experiment with isolation of virgin females showed that the cells degenerate in virgin females as well.

Inheritance

The character is recessive: when d_g females are outcrossed to males of other stocks the F_1 females have never shown cell-degeneration. At the time the character was found some work was done with it and a stock had been built up which contained besides d_g the 2 second-chromosome mutants, curved wings (*c*, locus 75.5) (BRIDGES AND STURTEVANT

1914) and cinnabar eyes (c_n, locus 57.5) (CLAUSEN 1924). This work had shown that the mutant was probably due to a factor in the second chromosome. To get some data on this according to the standard Star Dichaete method, a c_n d_g c female was outcrossed to a Star Dichaete (*SD*) male and F_1 *SD* males backcrossed to females of the c_n d_g c stock. It was found, however, that Star in this cross was constantly overlapping the normal type; the characteristic roughness of Star eyes was often unseen but, as was found later, the Star flies could be picked out by the smaller size of the eyes. The first backcross gave $28 d_g$: 216 non-d_g. It was suspected, however, that some of the flies classified as non-d_g were genetically d_g, which later experiments proved to be true. The low percentage of d_g flies was probably due to the fact that classification had been made on too young individuals.

After selection for extreme type of degeneration had been carried on for some generations, females and males from the d_g stock were outcrossed to wild males and females, and F_1 individuals from each kind of cross were mated together in 2 mass cultures and 2 single pair cultures. The results of the F_2 are given in table 2.

TABLE 2

Female offspring from the mating of F_1 individuals heterozygous for c_n d_g c.

CULTURE NUMBER	+++	$c_n d_g c$	$+ d_g c$	c_n ++	++c	c_n +c
35	65	14	1	9		3
36	39	7	3			1
Total	104	21	4	9		4
F_2 mass	155	50	3	5	4	13
Total F_2	259	71	7	14	4	17

In this, as in the following tables the symbols standing for the various mutant genes and + for their corresponding wild-type allelomorphs are used to designate the phenotype of the classes. Thus, +++ is used to designate individuals which are normal as regards the 3 mutant characters, cinnabar eyes, d_g and curved wings; the c_n d_g c class comprises the individuals which show all 3 mutant characters; whereas the $+d_g$ c class includes the individuals which show d_g and curved wings, but not cinnabar eyes. The ratio of non-d_g:d_g flies in the F_2 is 270:102. On the basis of one factor difference the expected numbers would be 279:93; so the results agree with the assumption that cell-degeneration depends on a single recessive factor. It is clear that this factor is carried both by males and females although it expresses itself phenotypically only in the latter. As

to the location of this factor, the results show that it is located in the second chromosome, somewhere near curved. To determine the locus of d_g females heterozygous for c_n d_g c were backcrossed to males homozygous for c_n d_g c.

The results from this cross are listed in table 3.

TABLE 3

Female offspring from backcrossing females heterozygous for c_n d_g c to c_n d_g c males.

CULTURE NUMBER	+++	$c_n d_g c$	$+d_g c$	c_n++	$c_n d_g +$	$++c$	c_n+c	$+d_g+$	TOTAL
52	52	12	1	12					77
53	63	34	2	4			2		105
54	24	20	1	3			3		51
55	13	7		1					21
56	63	34	6	8					111
58	22	14	2	7			3		48
59	22	12	4	3			1	2	44
60	34	28	3	8		1		1	75
61	39	20	5	3	2		1		70
88	48	34	5	7		1	7		102
89	54	30	3	4		1			92
90	61	71	11	8		1		1	153
91	112	135	18	16			1		282
92	59	62	9	4		1	1		136
Total	666	513	70	88	2	5	19	4	1367

From the results it is clear that the factor d_g is very near to c. In the class c_n+c there is an excess of individuals as compared with the corresponding crossover-class $+d_g+$. This is probably due to the fact that some of these individuals are really c_n d_g c in which the abnormality has failed to develop. If we compute the crossover value d_g-c only on the basis of the crossover-classes showing degenerated spermathecae, we find a value of 1.0 percent. The order of the genes and the relation between single and double crossing over will be discussed later, taking into account data from all the backcrosses.

There is in this cross a deficiency of 23 percent in the class c_n d_g c which may be due to a lower viability of the triple mutant type as compared with the wild-type. To get correct crossover values in cases with a notably lowered viability, one has to make *balanced viability crosses*, that is, different combinations of factors are used in crossing. Besides the cross in table 3 one further backcross was made: $c_n++/+d_g$ c ♀ ♀ $\times c_n$ d_g c ♂ ♂. To obtain females of this constitution c_n d_g c females were outcrossed to wild males and an F_2 was raised. F_2 d_g c individuals were mated together

and F_3 d_g c females were mated to males of cinnabar stock. Wild-type females from this cross, with the constitution $c_n++/+d_g\ c$ were mated to $c_n\ d_g\ c$ males. The results from these matings are given in table 4.

In this cross there is no deficiency of the d_g classes in relation to the non-d_g classes; the same holds true for the F_2 (table 2) and there is no

TABLE 4

Female offspring from backcrossing c_n++, $+d_g\ c$ females to $c_n\ d_g\ c$ males.

CULTURE NUMBER	$+d_g\ c$	c_n++	$c_n\ d_g\ c$	$+++$	c_n+c	$+d_g+$	$c_n\ d_g+$	$++c$	TOTAL
81	23	19	5		2			1	50
82	26	31	4	4			1		66
83	17	26	6	4	2			2	57
84	48	35	7	3					93
85	32	23	5	2			1		63
86	13	9	1	3	1				27
87	30	25	7	4	1				67
Total	189	168	35	20	6		2	3	423

significant deviation in another later backcross (table 5). It is probable, therefore, that the large deficiency in the first backcross (table 3) is not due wholly to lower viability of the d_g class, but is rather due to the fact that the females had to be kept for many days, and it was noted that there was sometimes a heavy loss of females during this time. In the later experiments this was avoided through a better method of keeping the females. The general impression is that the d_g stock is of good viability, although the $c_n\ d_g\ c$ type is not equal to the wild-type, which is not an unexpected condition in any stock containing 3 mutant genes. The d_g flies in table 4 give a crossover value $d_g - c$ 0.9 percent.

The next step in the location of d_g was to find a gene nearer to c and d_g than c_n; such a gene is Lobe^2 (L^2 locus 72.0) a dominant factor which has the effect of reducing the eyes. A Lobe^2 male was mated to a $d_g\ c$ female and F_1 Lobe$_2$ females were mated to $d_g\ c$ males. The results of this backcross are given in table 5.

The crossover value between d_g and c, basing the calculation only on the d_g classes is 1.2 percent.

The data do not allow a safe decision as to whether the genic order is $c_n\ d_g\ c$ or $c_n\ c\ d_g$, though the evidence is slightly in favor of the order $c_n\ d_g\ c$. There seems to have been in the experiment a low percentage of crossing over between c_n and c, 11.9 and 15.5 as compared with the standard value 18.0. There is further a high percentage of double crossing over. Although the amount of crossing over is thus somewhat abnormal

it is safe to conclude that d_g lies within about one unit to the right or left of curved (75.5 ± 1.0).

TABLE 5

Female offspring from backcrossing L^2++/+d_g c females to d_g c males.

CULTURE NUMBER	L^2++	+d_g c	+++	$L^2 d_g$ c	L^2 + c	+d_g +	$L^2 d_g$ +	++c	TOTAL
66	15	16					1		32
67	66	41	5					3	115
68	55	55	4					1	115
69	65	54	2	3	1			1	126
70	37	25	1		1		1	1	66
71	44	47	1	1		1		1	95
72	52	57	2	3	2			3	119
73	31	26	1	2	1	1			62
Total	365	321	16	9	5	2	2	10	730

TABLE 6

A summary of the 3 backcrosses, including only the d_g classes, on which basis the computations of the crossover percentages are made.

COMBINATIONS	−−	+−	−+	++	TOTAL	PERCENTAGE CROSSING OVER						
						c_n–d_g	d_g–c	c_n–c	double c_n–d_g–c	L^2–d_g	L^2+c	double L^2–d_g–c
c_n d_g c/+++	513	70	2	4	589	11.9	1.0	12.2	0.6			
c_n++/+d_g c	189	35	2	0	226	15.5	0.9	16.4	0.0			
L^2++/+d_g c	321	9	2	2	332		1.2		0.6	3.3	3.9	0.6

THE HEREDITARY BASIS FOR SUPERNUMERARY SPERMATHECAE

During the study of inheritance of the cell-degeneration it was necessary to dissect a large number of females. Some of the cultures were found to have many females with three spermathecae instead of two, which is the normal number.

The form and function of the spermathecae has been described by NONIDEZ (1920), while STURTEVANT (1926) has examined the intraspecific variability as to number of spermathecae. He examined specimens from wild stocks of *D. melanogaster*, *D. simulans*, F_1 hybrids between these two, *D. funebris*, *D. imigrans*, *D. busckii*, *D. repleta* and found two specimens with three spermathecae, all the others having two. The number of spermathecae thus seems to be a constant character and in the cases examined the species belonging to the same genus show the same number of spermathecae.

STURTEVANT (1926) describes a stock of *D. melanogaster* which showed a higher percentage of flies with three spermathecae and from which he

established a race in which from 25 to 75 percent of the females had three spermathecae. The genetic analysis has so far indicated that at least two factors are involved in the production of the extra spermatheca in this race.

In our d_g stock most of the individuals with three spermathecae had three of equal size; however, in some cases one was smaller than the other two. In individuals with two spermathecae one was sometimes larger than the other and often constricted. Occasional flies with four spermathecae were also found.

During the course of the investigation of d_g selection for three spermathecae was also carried on by always choosing flies from the cultures showing the highest percentage of three spermathecae. In a short time a stock was obtained which appeared to be homozygous for this new character. Two cultures counted at this time gave 205 with three spermathecae to 10 with two spermathecae. This stock was continued for several generations by brother-sister mating with no attention paid to the number of spermathecae. Then two cultures were again counted, giving 293 with three spermathecae to 4 with two spermathecae, which is 98.6 percent with three spermathecae.

The apparent ease with which a homozygous race was established indicated that the genetic basis for this character was perhaps simpler than in the case reported by STURTEVANT. It was, therefore, decided to attempt to determine the genetic basis for the inheritance of three spermathecae in this stock. The determination of the number of spermathecae was always made by dissecting the flies, a fact which in itself limits the number of flies which can be examined. Females from the three spermathecae (s_p^3) race were crossed with Star Dichaete (SD) and the F_1 SD ♂ ♂ were backcrossed to s_p^3 ♀ ♀, producing the following classes and numbers of female progeny.

$SD+$	SDs_p^3	$S++$	$S+s_p^3$	$+D+$	$+Ds_p^3$	$+++$	$++s_p^3$	total
81	—	78	1	45	2	66	32	305

The flies that have S or D or both do not develop s_p^3 (with the exception of 3 flies). This, therefore, indicates that there are in the second and third chromosomes recessive genes that contribute to the production of the third spermathecae. Since only a portion of the non-Star and non-Dichaete individuals show three spermathecae there must also be segregation in one other pair of chromosomes. If there were no other genes involved in the production of s_p^3 than those in the second and third chromosomes, then all the non-Star non-Dichaete flies should be s_p^3.

The segregation observed in the non-Star non-Dichaete flies cannot be due to a gene in the X-chromosome, because all the females of this culture have the X-chromosomes from the s_p^3 stock. Therefore, the segregation in non-Star non-Dichaete flies is probably due to a recessive gene in the IV-chromosome (table 7). A definite proof of the existence of such a gene in the fourth chromosome can only be delivered through linkage tests with a fourth chromosome mutant. This would require additional mutant stocks and additional time neither of which was available.

TABLE 7

Comparison of observed and calculated results on the basis that s_p^3 is due to the simultaneous action of three recessive genes, one in each of the II, III and IV chromosomes.

CHARACTER	*SD*	*S*	*D*	+	s_p^3	TOTAL
Observed numbers	81	78	45	66	35	305
Calculated numbers	*76*	*76*	*76*	*38*	*38*	*304*

The deviation from the 1:1 ratio in the Dichaete and normal classes cannot readily be explained, but it is probably due at least in part to difficulties in recognition of characters. The culture also involved curved, a wing character brought in by the d_g stock, which may have in some way interfered with classification. However, if we consider the segregation of s_p^3 and non-s_p^3 on a three chromosome (II, III and IV) basis the correspondence of observed and calculated numbers is very good.

Observed 270.0 non s_p^3 to 35.0 s_p^3
Calculated 266.7 non s_p^3 to 38.1 s_p^3

These results do not exclude the possibility of essential genes for s_p^3 being in the X-chromosomes; for these flies were all homozygous for these chromosomes. That the sex chromosomes are not involved, however, is indicated by the results of another backcross in which F_1 ♀ ♀ were used.

TABLE 8

Results of crossing ♀ ♀ *of the constitution* $\frac{+\ c_n\ d_g\ c\ s_p^3}{S++++}\ \frac{+s_p^3}{D+}$ *with* $\frac{+\ c_n\ d_g\ c\ s_p^3}{+\ c_n d_g\ c\ s_p^3}\ \frac{+s_p^3}{+s_p^3}\ \frac{s_p^3}{s_p^3}$ ♂♂.

Classes	*SD*	*S*	$c_n D$	$c_n c$	$c_n c s_p^3$	*D*	Sc_n	*Sc*	Total
Number	29	13	35	22	12	20	3	1	
Classes	$S c_n D$	+	c_n	c	s_p^3	$D s_p^3$	$c_n D s_p^3$	$SD s_p^3$	
Number	1	13	5	1	1	1	1	2	160

A ♀ from a cinnabar- d_g- curved- s_p^3 stock was crossed with a SD♂ and F_1 SD ♀ ♀ backcrossed to males of the c_n- d_g- c- s_p^3 race. Three backcross cultures gave the results in table 8. In this table curved-Dichaete flies are listed with the Dichaete.

If we consider the ratio of s_p^3 to non-s_p^3 we get, as before, evidence of a segregation in only 3 chromosomes.

Observed 143 non-s_p^3:17 s_p^3
Calculated 140 non-s_p^3:20 s_p^3

The male and female backcrosses thus give similar results showing that the sex chromosomes do not carry genes essential for the s_p^3 character. The conclusion is reached, therefore that the extra spermathecae in this race is dependent upon recessive genes in the second, third and fourth chromosomes. The appearance of Star s_p^3 and Dichaete s_p^3 flies may mean that in some cases the third spermathecae can be produced when either the second or third chromosome genes are in a heterozygous condition if the other two sets of genes are in a homozygous condition.

No definite evidence has been obtained relative to the linear location of any of the three s_p^3 genes. On the suggestion of DR. STURTEVANT females from cultures of SD from the stock used in this analysis were dissected to determine whether any of them carried 3 spermathecae. Two single pair cultures and one mass culture gave 73 SD:39 $S+$females all with 2 spermathecae; no $+D$ and $++$females. A probable explanation of this result is that a lethal has arisen by mutation in the second chromosome allelomorphic to the S gene, thus producing a balanced lethal stock breeding true for S. A culture, segregating for S, gave the result 12 SD:8 $S+$:7$+D$:1$++$females, all with 2 spermathecae. In all 140 females were examined, all had 2 spermathecae. It is not likely, therefore, that there are in the SD stock used in the analysis of the supernumerary spermathecae any genes which would interfere with the analysis.

As has been mentioned, the number of spermathecae, is a fairly constant character within a species, and as a rule uniform for all the species of a genus. It is, therefore, of interest to find such a character arising as a mutation in one species, giving rise to a race breeding true for a number of spermathecae, different from that which is characteristic of the Drosphilidae.

The genetic basis of this character provides an interesting side light on the problem of organic evolution. Against the theory of evolution through the selection of small independent mutations, it is urged as a main difficulty, that complex organs which must depend upon the interaction of many genes cannot be thought to arise through a series of quite indepen-

dent random mutations. So far all evidence indicates random mutation in *D. melanogaster*. It is reasonable to assume that it is the case here. Three mutations have occurred, each of which apparently has no influence upon development, but which together interact to cause the development of a complex organ, an extra spermatheca. This tends to show that the criticism of the theory, that mutations form the basis of organic evolution, may not have the importance often attached to it.

SUMMARY

1. In this work is studied a hereditary cell-degeneration on the spermathecae of the females of *Drosophila melanogaster*. It arose as a mutation in a stock of curved flies and has been proved to be dependent on one single recessive factor located in the second chromosome near curved 75.5 ± 1.0.

2. The abnormality appears only in adult females some time after emergence and consists in a deposition of a dark brown pigment in the epithelial layer of the spermathecae; in highly degenerated spermathecae the pigment forms a complete bag around the spermathecae, and is visible as conspicuous black spots on the abdomen of the females.

3. In pure stock nearly all the females show the cell-degeneration, but the degree of degeneration is variable and has been shown to be influenced by the amount of food in the cultures, such that with a smaller amount of food there is higher degree of degeneration.

4. The viability and fertility of the d_0 females are good.

5. During the cell-degeneration investigations a stock was established which was homozygous for the new mutant character, supernumerary spermathecae, that is, 3 instead of the normal number 2.

6. A genetic analysis showed this character to be dependent upon recessive factors in the second, third and possibly fourth chromosomes.

7. It is pointed out that this character has an interesting bearing on the problem of organic evolution.

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A FURTHER STUDY OF THE SO-CALLED MUTATION AT THE BAR LOCUS OF DROSOPHILA

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INTRODUCTION

An earlier study (STURTEVANT 1925) of the "mutations" at the bar locus of *Drosophila melanogaster* led to the conclusion that these mutations are due to unequal crossing over at the bar locus. Reverted bar is no-bar, having no allelomorph at the bar locus; double-bar (the ultra-bar of ZELENY) has two bar genes lying next each other in the linear series of genes.

The present paper gives the results of a few experiments designed to clear up certain points left doubtful by the earlier study.

TIME OF OCCURRENCE OF BAR MUTATION

ZELENY (1921) recorded five cases in which more than one reversion appeared in a single culture, and concluded from this evidence that reversion probably occurs, sometimes at least, in oogonial cells. Since the five cultures in question were not from individual females they do not prove that several reversions may be produced by a single mother, but they are at least suggestive. If, following this suggestion, one assumed that unequal crossing over leading to bar reversion occurred at some stage earlier than the maturation divisions, it would be expected that normal crossing over between forked and fused would occur after it. The result should be that about 3 percent of the "mutations" at the bar locus would appear to be unaccompanied by forked-fused crossing over—and the data on record show a small number of such exceptional cases. A further consequence, open to experimental test, should be that bar reversion would not interfere with crossing over in neighboring regions.

[1] Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

Accordingly an experiment has been carried out with females homozygous for infrabar (used instead of bar in order to make the separation for eye-color easier), and heterozygous for garnet-2 forked and fused (see figures 1 and 2).

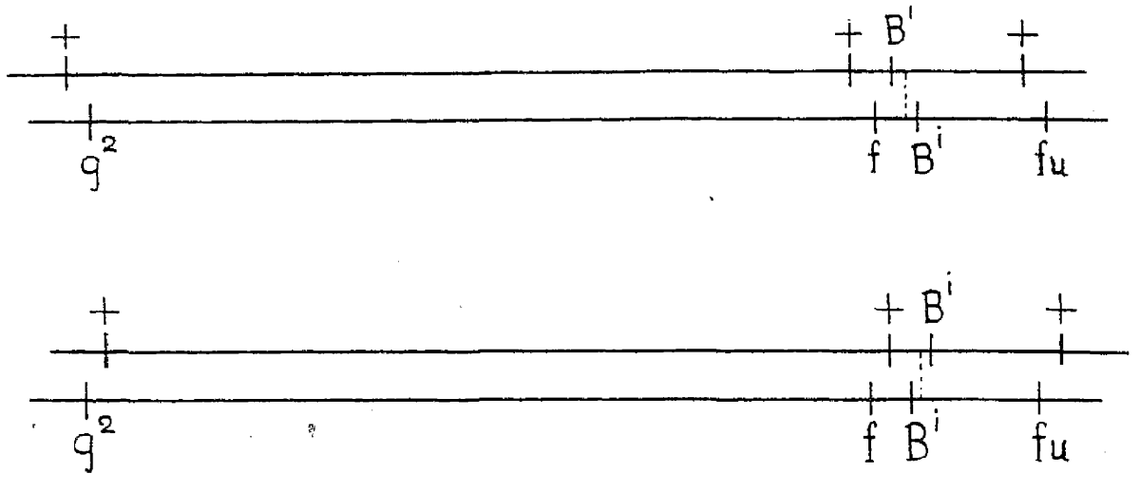


Figure 1 (above) and Figure 2 (below).

TABLE 1

$$\textit{Offspring from } \frac{B^i}{g^2 f\, B^i f_u} \text{♀} \times g^2 f\, B^i f_u \text{♂}.$$

B^i									B^iB^i				+			
0		1		2		1,2		Total	♀*		♂		♀*		♂	
+	$g^2 f f_u$	$f f_u$	g^2	f_u	$g^2 f$	f	$g^2 f_u$		f_u	$g^2 f$	f_u	$g^2 f$	f_u	$g^2 f$	f_u	$g^2 f$
19536	14939	2351	2678	574	647	4	7	40736	4	2	2	6	4	7	11	4

* The "mutant" females were, of course, all heterozygous for B^i, received from the father.

A small series (1647 individuals) from $\frac{f\, B^i}{g^2 \quad B^i f_u}$ also gave a reversion ($f\, f_u$) that was not a $g^2 f$ crossover. In the two series there is a total of 41 "mutant" individuals, all of them crossovers between forked and fused, but none of them garnet-2 forked crossovers. There are 5040 garnet-2 forked crossovers among 40736 unmutated offspring of the table, or 12.4 percent, in good agreement with the 12.1 percent recorded by MORGAN, BRIDGES and STURTEVANT (1925).

If mutation and garnet-2 forked crossing over are unrelated, the chance that none of the 41 mutant individuals should be a garnet-2 forked crossover is therefore $(1-.124)^{41}=.004$, or 1 in 250. One may safely conclude that the unequal crossing over that gives rise to mutation at the bar locus

interferes with crossing over in the garnet-2 forked region, just as does ordinary crossing over between forked and fused, and hence that the unequal crossing over occurs at the same time as ordinary crossing over.

There must, therefore, be other explanations of the two relations that led to the experiment. The occurrence of mutations not accompanied by forked fused crossing over is open to two interpretations, as previously pointed out (STURTEVANT 1925). Crossing over between sister strands is a possible explanation, but if it occurred at all would be expected to give many more apparent non-crossover mutations than have been observed. The most probable view seems to be that all such records are due to experimental errors. It is to be noted that none occurred in the present series of experiments.

As pointed out above, ZELENY'S data do not prove that more than one mutation was produced by a single mother in any case. However, in the present series there were four separate cultures in which a single mother produced two mutant offspring, as follows:

Culture	*Mutant offspring*
21794	$B^iB^if_u$, $g^2fB^iB^i$
22254	f_u, g^2f
23611	f_u, f_u
23898	$g^2fB^iB^i$, g^2f

Examination of these data shows that in three of the four cases the two individuals cannot have been produced by one crossing over.[1] In the first case, for example, the crossing over that produced a $B^iB^if_u$ chromosome would also give a g^2f (figure 1)—but could not give $g^2fB^iB^i$, the combination actually recovered. It follows that the production of more than one mutant offspring by a single mother must be due to the occurrence of more than one unequal crossing over in that mother. Whether or not such coincidences are more frequent than would be expected with a random distribution can hardly be determined without the collection of a vast amount of accurate data.

RELATIVE NUMBERS OF REVERSIONS AND DOUBLE-BARS

On the hypothesis of unequal crossing over a homozygous bar stock should produce equal numbers of double-bar and wild-types "mutants," but all observers have found distinctly more wild-type than double-bars.

[1] Unless by chance one of the rare "normal" forked fused crossovers followed the mutation in one egg and not in the other in each case. The chance that this should have happened in all three instances is negligible.

I have suggested (STURTEVANT 1925) that this is due to two factors—lower viability of double-bar and greater likelihood of overlooking mutant individuals. The viability factor can be directly measured. Using the inbred stocks prepared for the facet counts recorded in my earlier paper, I have made counts from double-bar over round mated to round and to bar. A small series gave 60 double-bar over bar females to 82 bar over round, and 126 double-bar males to 161 round males. Equality is expected in each case, and the deviations are due to differential viability. It follows that, in the combinations met with as mutant individuals in a homozygous bar stock, double-bar has a viability about 75 percent that of round.

ZELENY (1921) records the production of 52 reversions and 3 double-bars among 85008 specimens examined. In addition there were at least 5 double-bars that were not tested, so that we may take 52:8=6.5:1 as the observed ratio. ZELENY argues that there were probably very few mutant individuals overlooked—probably only a few of the double-bar females. I obtained (STURTEVANT 1925) 8 reversions and 2 double type from homozygous bar, 18 reversions and 3 double types from homozygous infrabar, and 2 reversions and 3 double types from bar over infrabar—a total of 28:8=3.5:1. This is somewhat closer to the expected equality than ZELENY's 6.5:1, but the experiments here reported for homozygous infrabar gave 27:14=1.9:1. This is still a definite excess of reversions, over what can be accounted for by differential viability, especially as the viability difference is less for double-infrabar than for double-bar, but more carefully conducted experiments and more experience having lowered the ratio from 3.5:1 to 1.9:1, I feel confident that there is still room for technical improvement sufficient to remove the remaining discrepancy.

POSITION EFFECT

It was shown (STURTEVANT 1925) that two bar genes lying in the same chromosome are more effective in reducing facet number than are the same two genes when they lie in different chromosomes. It seemed possible that such a relation might also hold for non-allelomorphic genes, but the one test, now to be described, has given a negative result.

The two dominat genes delta and hairless lie 3 units apart in the third chromosome (BRIDGES and MORGAN 1923). Hairless causes a reduction in bristle number, and delta partially counteracts this effect. These genes were selected for study because they lie close together, and because bristle number is convenient for quantitative work. An inbred strain was established, in which repeated brother-sister matings and repeated use of crossovers between delta and hairless made it probable that modifiers were

uniform. Matings were made in parallel, delta×hairless and delta hairless×wild-type. All offspring from these matings were reared at 26°C. The results are shown in table 2.

TABLE 2

Number of dorsocentrals.

Mating	3	4	5	Total	Mean
Δ X H	5	95	0	100	3.95
Δ H X +	10	167	1	178	3.95

While the numbers here are small, the exact agreement in mean number of dorsocentrals makes it seem certain that there is no position effect.

As pointed out in my earlier paper, the pairing of homologous chromosomes that occurs in somatic divisions in Drosophila suggests that there may be a position effect normally present of such a nature that allelomorphs reinforce each other's effects. Such an effect was offered as an explanation of the different dominance relations observed in triploids and in cases of translocation (MORGAN, BRIDGES and STURTEVANT 1925). A new set of facts makes this interpretation now seem improbable. I have shown (STURTEVANT 1926) that the C_{III} present in ebony and other stocks is due to the inversion of a large section of the third chromosome, and there is now evidence that the $C_{II\ L}$ of the curly stock and two other C_{III}'s are due to similar inversions. In flies heterozygous for such inversions the position relations of many genes are greatly changed, but no difference in somatic appearance has yet been detected in such flies. It follows that changes in position of this order are not usually of developmental significance. One may conclude that the association of the two elements in double-bar is much more intimate than the association between identical loci in homologous chromosomes, and that the "position effect" perhaps rests on something more than mere closeness together.

FACET COUNTS OF DERIVED TYPES

Facet counts recorded (STURTEVANT 1925) for a reversion from bar and one from infrabar suggested that these derived rounds might be different from the wild-type, since both strains gave lower facet numbers with bar-infrabar (one of them also with double-bar and in males) than did wild-type. The inference that there is a wild-type allelomorph of bar, absent in reversions, and having an effect on facet-number opposite to that of bar, was further tested by studying an infrabar from bar-infrabar over round, and a bar from double-bar over round. Both of these derived types when tested against double-bar, gave somewhat larger facet-numbers than the controls (original infrabar and original bar, respectively). It was suggested

that perhaps the derived types carried wild-type allelomorphs, which partially counteracted the effects of the bar and infrabar genes. However, the differences were small in all these experiments, and it was emphasized that more tests were needed.

Several new derived types have now been studied, and have not borne out the assumption of a wild-type allelomorph.[2]

Two new infrabars, both from double-infrabar over reverted infrabar, would have been expected to give the same results as the old infrabar, even if there is an effective wild-type allelomorph—since such an allelomorph should not be present in either the old or the derived types. The result of a test against double-bar was: control (old infrabar) 35.7 facets, new infrabar 33.6 and 35.4 facets, respectively (individuals counted, 51, 30, and 30). The expectation is clearly correct; there are no significant differences.

A new reverted double-infrabar was tested, and at the same time the reverted infrabar of the earlier experiments was used. These and wild-type were tested against double-bar. Forty females of each type were counted, with these results: wild-type, 43.8; reverted infrabar, 44.4; reverted double-infrabar, 43.2. The reverted infrabar differs from wild-type in the opposite direction from the earlier test, and none of the differences here seem significant.

Two new bars, both from double-bar over round, were tested in three ways, as shown in table 3.

TABLE 3

Facet counts.

	OVER B^iB^i		HOMOZYGOUS ♀		MALE	
	n	*M*	*n*	*M*	*n*	*M*
Bar (control)	45	37.4	25	61.8	30	69.8
First derived bar	45	38.2	25	54.4	30	67.6
Second derived bar	45	35.4	25	49.6	60	61.0

Both of the derived types give lower facet numbers than the control, and in the case of the second derived type this difference is probably signi-

[2] The reverted double-infrabar and the first derived bar arose in the inbred forked stocks that were used in the earlier study. The other derived types were crossed at least five times to these stocks before counts were made. All the other stocks used in the present experiments were the same as those described in the earlier paper. The arguments for supposing modifiers to be alike are the same as in the earlier experiments. The temperature was 25° ± 1°.

ficant—but the difference is in the opposite direction from that found in the earlier experiments.

The conclusion seems to be justified that the observed differences are due to uneliminated modifiers or to chance environmental differences. In any case the new results do not at all support the hypothesis of a wild-type allelomorph affecting facet-number.

The three homozygous bar types recorded above averaged 55.3 facets; a double-bar over-round series of 40 females reared at the same time gave a mean of 43.8. This gives a position effect of the same kind as the data in the earlier paper, but rather less in extent.

REVERTED INFRABAR AND CROSSING OVER

Reverted bar or infrabar may be considered as a deficiency which is so short that it does not have the lethal effect of most deficiencies. It has been shown by BRIDGES (1917, 1919) and MOHR (1923) that females heterozygous for the usual type of deficiency give no crossing over in the section covered by the deficiency. This fact suggested another test of the relation between reverted bar and wild-type. It has been shown (STURTEVANT 1925) that any double-type over wild-type gives about 0.1 percent of single-type, the mutant individuals always being forked-fused crossovers. That is, in such heterozygous females there is crossing over between the two elements of the double-type. A test has now shown that reverted infrabar gives the same result as normal with double infrabar (table 4).

TABLE 4

Offspring from $\frac{B^iB^i}{f\,r_{ev}f_u}$ ♀ × $f\,r_{ev}f_u$ ♂.

0		1		2		TOTAL	MUT.
B^iB^i	ff_u	f_u	fB^iB^i	$B^iB^if_u$	f		fB^i
1474	1415	1	3	35	42	2968	2

IS A WILD-TYPE ALLELOMORPH OF BAR NORMALLY PRESENT?

The results reported in this paper indicate that reverted bar and reverted infrabar do not differ from wild-type in their effect on facet-number or in their crossing over relations in heterozygotes with double-types. No other kind of test appears to be available, so the simplest conclusion is that ordinary stocks carry no allelomorph of bar. This is consistent with the fact that the change from round to bar has been detected only once in *D. melanogaster*, and no similar mutation has occurred in any other species.

One may surmise that the stock that gave rise to the original bar mutation had some sort of allelomorph already present—though other assumptions may also be made. It should be noted that, if a normal allelomorph were present in a stock and underwent unequal crossing over, as bar and infrabar do, the only stable condition would be that in which no bar allelomorph was present. While unequal crossing over does not alter the number of bar allelomorphs present in a stock, any strain without such allelomorphs will breed true, while any strain in which they are present will still give occasional individuals lacking them. If it be granted that Drosophila populations show great fluctuations in numbers, and often are reduced to very few individuals—as is very probable from their habits—then it would, in fact, be expected that unequal crossing over would have reduced most strains to the "no-bar" condition.

SUMMARY

1. "Mutation" at the bar locus interferes with crossing over in the neighboring garnet-forked interval, indicating that unequal crossing over at the bar locus occurs at the same time and by the same mechanism as normal crossing over.

2. Homozygous bar or infrabar stocks probably give equal numbers of reversions and of double-types; but the latter are somewhat less viable and much less easily detected, so that reversions are found more often. Improved technique has, however, decreased the observed difference.

3. The dominant mutant types Delta and Hairless show no "position effect" on bristle number—that is, $\Delta H/+$ and Δ/H do not differ in bristle number. This and other evidence suggests that relative position of genes is not usually significant in development—though new data agree with old in showing that it is significant in the case of bar.

4. New data indicate that reverted bar (or reverted infrabar) is not different in facet-number from wild-type, and that derived types in general show no effects of their past history.

5. Double-infrabar over reverted infrabar gives infrabar.

6. The last two points indicate that reverted bar (or reverted infrabar) is not different from wild-types, and the conclusion is drawn that ordinary wild-stocks carry no bar allelomorph.

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LINKAGE STUDIES WITH THE TOMATO. II. THREE LINKAGE GROUPS

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INTRODUCTION

In the first study of this series (MACARTHUR 1926) nine of the most distinct qualitative characters of the tomato were tested in 24 different combinations, two at a time, for evidence of linkage. F_2 populations gave definite proof of the association of four of the controlling genes in one compact linkage group, and strong evidence of the independent assortment of the five remaining genes. There were thus established with reasonable certainty one linked group and basic "markers" for five others. This main conclusion still accords completely with all the well tested genetic data in the literature, and includes and confirms whatever linkages of qualitative characters have been determined by others (HEDRICK and BOOTH 1907; CRANE 1915; and LINDSTROM 1924–1927), but does not confirm the suspected linkage, between factors for yellow foliage and bilocularity, mentioned by PRICE and DRINKARD (1908) and JONES (1917).

The much-needed support of adequate backcross data, involving the same factors, has now been in large measure supplied. This new data, so far as it is merely confirmatory, is being published elsewhere, and will be cited in this paper only when concerned in some direct and essential way with the linkages to be described.

The internal relations existing between the members of the first linked group may now be stated more accurately, and the backcross recombination values given for all the possible pairings of factors. Two additional linkage groups, newly discovered with the help of mutant characters not hitherto employed in such work, are also described.

This account may be regarded in and for itself, but especially as foundation-building for succeeding studies on the most quantitative genetic factors, on mutation nature and frequency, and on the special genetics of types with changed chromosome numbers.

THE FIRST LINKAGE GROUP

Genes Dd, Pp, Oo (=P_r p_r), and Ss

The genes at the four loci known to be occupied in the first established group are mostly well known ones, already described (see especially PRICE and DRINKARD 1908; CRANE 1915; LINDSTROM 1924–1926; and MACARTHUR 1926). They are the differential factors controlling contrasting types of:

1. Stature. Tall (*D*) vine is dominant over dwarf (*d*). Nearly all, if not all, of the numerous dwarf varieties (included in the "Validum" subsection of the species by BAILEY 1896, p. 479), with their characteristic short, stiff, thick-set, tree habit and invariably associated dark green, rugose foliage, are probably derived ultimately from the same original "Laye Upright" mutant (VILMORIN-ANDRIEUX 1920), which appeared in a French garden about 1850. Their intercrosses have yielded only dwarf type plants, though these often differ interestingly in degree. But dwarf is to be distinguished from one "brachytic" sort, bearing normal foliage.

2. Fruit skin pubescence. Smooth (*P*) is dominant over peach (*p*).

3. Fruit elongation. Short (P_r or *O*) is partially dominant over elongate (p_r or *o*). To explain the multitude of varied and true-breeding shapes of tomato fruits certainly requires that one invoke the assistance of a considerable number of factors, factor combinations, and factor interactions. Prominent major factors, whose effects on shape have been isolated and identified more or less satisfactorily, are: a pair controlling the chief differences between non-fasciation and fasciation and thus affecting both locule number and shape; and two or three allelomorphs controlling degrees of elongation of the fruit. The exact mutual interrelations of these two sets of gene effects are not always entirely clear, but most of the difficulty presumably lies in our ignorance as to the number, manner of action, and precise effects of other modifying major and minor shape factors.

In any case there is no doubt that to the first linked group belong the

factors regulating relative length of fruits, that is the width/length ratio, as it varies from the somewhat flattened (oblate and peach), through the nearly spherical sorts (as in Apple, and Cherry), to those of more elongate contour (oval, Plum, Fig, Pear, etc.). Whether the factor causing constriction of neck in pear and calabash forms also belongs here, or is merely a modifier with another locus, should be settled by crosses already made. For the present purpose a single major elongation factor ($p_r = o$) may safely be considered as the factor characteristic of all of the related fruit shapes grouped by BAILEY (1896) in the "Pyriforme" subsection of the species; as such it is partially recessive to one or more allelomorphs ($P_r = O$) in round and oblate sorts. These factors exhibit like linkage relations and values.

4. Inflorescence types. The usual simple cluster (S) is dominant over the compound, much-branched (s) type, carefully described and studied by CRANE (1915).

To the above should possibly be added rather uncertain and unlocated fruit size factors (LINDSTROM 1926b) and, even more doubtfully, a pair that may control resistance or susceptibility to western yellow blight (LESLEY 1926a).

Linkage relations within the first group

Tall-dwarf (*Dd*) and Smooth-peach (*Pp*) genes

The exceedingly close linkage (repulsion) shown by the total absence of the double recessive class in F_2 populations, numbering 677 plants, was noted again by LINDSTROM in a backcross generation, where it appears (1926b) that among 119 plants only four (3.4 percent) represented crossovers.

Tall-dwarf (*Dd*) and Round-elongate (pyriform) (P_r p_r or Oo) genes

Evidence for fairly strong linkage, with 11–20 percent crossing over was found in various F_2 populations in both coupling and repulsion phases. The shape phenotypes were again classified, as in the earlier work, by caliper measurements in both axes and by weights (yet unpublished). The evidence for linkage was sustained by the occurrence among 257 backcross progeny of 31 recombinations (12 percent). Their relative frequency in the reciprocal backcrosses demonstrates further that approximately equal crossing over takes place in microsporogenesis (13.2 percent) and in macrosporogenesis (11.7 percent) (table 1).

TABLE 1

Data from back crosses showing repulsion between d and o genes.

BACKCROSSES ♀ ♂	CLASSES				PERCENT CROSSOVERS
	Tall		Dwarf		
	Flat-round	Long-pear	Flat-round	Long-pear	
dd oo×F_1	5	28	18	2	13.2
F_1×*dd oo*	12	97	83	12	11.7
Total	17	125	101	14	12.06

By way of comparison, the similar more recent data of LINDSTROM (1927), obviously collected by use of the same criteria in classification, show a satisfactory agreement with 10.9 percent recombinations. Totals from both sources thrown together give 70 recombinations among the 616 plants of the backcross generation, that is, 11.4 percent crossing over in general, 11.9 percent in the male and 11.0 percent in the female.

Smooth-peach (*Pp*) and Round-long (pear) (*Oo*) genes

Ratios noted from 700 F_2 plants segregating for these genes suggest (Q = −.9) about 14–15 percent crossing over, allowing parity for the sexes. The F_2 findings are supported in essentials by backcrosses, which, however, point to an even stronger linkage (8.6 percent crossing over), since, among 280 plants, most bore smooth pear or peach round fruits, and in 24 cases only did new combinations appear (table 2).

TABLE 2

Backcross data showing linkage (repulsion) between p and o genes.

BACKCROSSES ♀ ♂	CLASSES				PERCENT CROSSING OVER
	Smooth		Peach		
	Round	Long	Round	Long	
pp oo×F_1	8	109	115	12	8.2
F_1×*pp oo*	4	14	18	0	11.11
Total	12	123	133	12	8.57

Round-elongate (*Oo*) fruit and Simple-compound (*Ss*) inflorescence genes

From the cross, Yellow Pear (*oo SS*)×Grape Cluster (*OO ss*) the F_1 hybrids bore practically round fruits in simple clusters, and the 259 F_2 progeny fell into classes showing about 20 percent crossing over in both sexes. Two hundred and ten backcross plants confirmed this value (table3).

TABLE 3
Data showing linkage between s and o genes.

GENERATION	CROSSES	CLASSES				Q VALUE	PERCENT CROSSING OVER
		Simple		Compound			
		Round	Long	Round	Long		
	(*repulsion phase*)						
F_2	Y. Pr.×Gr. Cl.	126	66	63	4	−.784	20
B. Cr.	*ss oo*×F_1♂	23	83	85	19		20
	(*coupling phase*)						
	CRANE's data (1915)						
F_2	W.I.×L.P.	91	16	11	13	.80	23

This determination of linkage value brings into line and explains reasonably what is apparently the last isolated case of linkage in the literature of tomato genetics—the hitherto unrelated results obtained by CRANE (1915) from his cross of Wonder of Italy with Lister's Prolific. The former variety bears oval fruits in compound clusters, the latter nearly spherical fruits in simple clusters; and CRANE states that the F_2 ratios "suggest partial coupling . . . . between the factor for simple inflorescence and that for short fruit" but that the numbers were too limited to estimate its intensity, or even to establish the linkage with certainty. But the association coefficient (Q = .8) and ratio fitting demonstrate that such a ratio is made logical if 20–23 percent crossing over occurs. Quite evidently the gene for long (pyriform) fruit and that for long (oval) fruit occupy the same locus, if indeed they be not identical.

Smooth-peach (*Pp*) and Simple-compound (*Ss*) genes

From the cross of Pink Peach (*ppSS*) with Grape Cluster (*PPss*) the smooth-skinned and simple-clustered F_1 plants have so far produced 195 F_2 individuals in the repulsion ratio: 104 *PS*:32 *Ps*:41 *pS*:7 *ps*, suggesting something over 30 percent crossing over. That this value is too high is indicated by the much more dependable backcross ratio; 184 plants from the double recessive F_2 ♀ ×F_1♂ were distributed in the proportion: 19 *PS*:71 *Ps*:68 *pS*:26 *ps*, the recombination value falling at 24.46 percent.

Tall-dwarf (*Dd*) *and simple-compound* (*Ss*) *genes*

The double recessive, dwarf compound (*ddss*), extracted from the earlier cross, when pollinated by the F_1 hybrid produced the repulsion backcross ratio: 21 *DS*:36 *Ds*:40 *dS*:9 *ds*, indicating 28.3 percent crossovers. But the considerable deficiency of the last class is to be noted.

Summary of crossover value

In table 4 are summarized from the sources mentioned above, the crossover values obtaining between the genes of the first linkage group.

TABLE 4

	d	*p*	*o*(p_r)	*s*
dwarf	..	3.4	11.4	28.3
peach		..	8.57	24.46
oval-pear			..	20.0
compound				..

The summaries (table 4 and figure 1) suggest that linkage group organization in the tomato resembles closely the classical Drosophila picture. Observed recombination percentages approximately correspond to map distances up to about 12 units; but fall short more and more for the greater distances, where double crossing over presumably occurs. Considering the best interpretation of the linkage values, it seems likely that the order of the genes in the chromosome is, therefore: *d*-*p*--*o*(p_r)----*s* and that the map loci stand at about 0.0, 3.4, 12.0, and 32+units respectively from the left end. But before stating these relations with the proper finality, further corroboration is desirable; hence the decision to await the maturing of results in several 3-point tests and the one 4-point test. Should these uphold the conclusions tentatively adopted, then the mapping of this chromosome will have been begun.

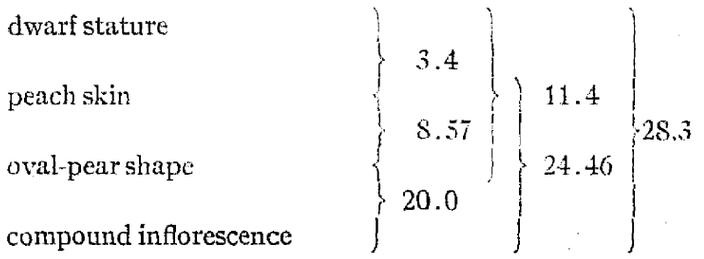


FIGURE 1.—Plan to show the probable linear order of genes of the first group and the observed percent of crossing over between them in their different pairings.

A SECOND LINKAGE GROUP

Description of genes Ll and Uu

The factors here involved are two simply mendelizing pairs, both concerned with chlorophyll coloration, one affecting the amount of the green pigment in general, the other the localized distribution of the color on the green fruits:

1. Color of foliage and immature fruits—*Ll* genes (heretofore designated as *Gg*). The normal full green color of foliage and young fruits (*L*) is dominant over the gradually yellowing, "lutescent" foliage (*l*) with its associated pale yellowish-white unripe fruits.

2. Distribution of green color over the immature fruits—*Uu* genes. The usual darker green stem-end coloration covering the basal third or fourth

of unripe fruits (*U*) is dominant over a mutant type found in one English variety, Devon Surprise, whose fruits have a perfectly uniform pale-green coloration from stem to stylar star (*u*). This lack of pattern, or self-colored pale condition, is a difference distinctly visible on green fruits of all sizes, is not masked or obscured appreciably by the presence of any other genes so far studied, even the pair *Ll*, and has no associated gene effects apparent in the color of ripened fruits or elsewhere in the plant.

Genetically this new condition is recessive to the normal in hybrids and is recovered pure-breeding in about 25 percent of the F_2 generation plants. The numerous crosses made with Devon Surprise have so far yielded an F_2 ratio of 914 plants bearing fruits with the dark butts to 297 with the uniformly colored fruits; and two sets of backcrosses have produced 166 dark: 152 uniform colored.

Genetic relations of Ll and Uu to other genes studied

That the genes, *Ll*, controlling foliage color, are independent in inheritance from the genes of the first linked group and from those controlling fruit flesh color (*Rr*), fruit skin color (*Yy*), fruit fasciation (*Ff*), and leaf shape (*Cc*) is indicated by data published (MACARTHUR 1926) and unpublished. Crosses and backcrosses of Devon Surprise with the linkage testers have demonstrated the same independent assortment between *Uu* and these genes; these negative data are being published in another place. In only one combination of genes, that is of *Uu* and *Ll*, was evidence of linkage obtained.

Linkage relations within the second group

In the exceptional case, where Devon Surprise (*uuLL*) was crossed with a linkage tester containing, among others the *UUll* genes, the normal type F_1 hybrids produced in the F_2 generation the significant ratio: 68 green foliaged with dark fruits: 33 lutescent with dark fruits: 22 green foliaged with uniform-colored pale fruits: 2 lutescent with uniform-colored pale fruits. The last two classes, those with self-colored pale fruits appear to have too low values, but whether correction is made for this or not the calculated coefficient of association gives the same figure, $Q = -.6845$, indicating a crossover percentage of 26+percent between *Uu* and *Ll* genes.

Since a supposed linkage between factors controlling foliage color (*Ll*) and those controlling locule number (*Ff* or others) is persistently, and apparently erroneously, cited in genetics texts, I offer here one sample of the abundant data, showing the complete independence of these character pairs. One cross involving these pairs has produced F_2 plants as follows:

218 green-foliaged with an average of 3.244 locules and 61 lutescent with an average of 3.25 locules. Nor is there any evidence of linkage between *Uu* and *Ff* genes, as would be expected if the admittedly very doubtful linkage based on the segregation of only 24 F_2 plants (PRICE and DRINKARD 1908) were real. But, until their experiment is repeated with the original varieties used I would not presume to imply that such an association does not exist, improbable though it now seems.

A THIRD LINKAGE GROUP

Description of genes Ff and Aa

The factors associated in this group are those controlling the widest differences in fruit fasciation (*Ff*), and a newly tested pair (*Aa*) controlling anthocyanin color development.

Fruit Fasciation

Non-fasciated fruits (*F*) of smooth outline and containing few cells, are dominant over fasciated fruits (*f*) of rough, irregular contour, containing usually 8–12 or more cells, some central in position; and when hybridized the F_2 populations segregate to give a simple 3:1 ratio. Never in our very numerous crosses have we found a 9:7 or other ratio indicating control of fasciation by two pairs of complementary factors such as WARREN (1925) reported.

Anthocyanin pigmentation

Usually the aerial portions of tomato plants, especially the stems and leaf veins, are distinctly purplish due to the presence of the sap pigment, anthocyanin. But an anthocyanin-less mutation (NORTON 1910) appeared in an inbred strain of Red Cherry tomatoes, as observed after its occurrence, by T. H. WHITE, when "a flat of seedlings produced a number of plants with entirely green stems." These green-stemmed plants bred true, and some of their purple sibs produced both purple and green progeny in a 3:1 ratio. Thirteen-year old seed of the mutant, kindly supplied to the writer, germinated well despite its age; thus reestablished, the green stem type behaved in crosses as a simple recessive to purple, and segregated pure in a ratio of 294 purple: 104 green plants. Winter-grown in the greenhouse, it is comparatively easy to distinguish heterozygote purples from pure purples, and these classify according to a 2:1 ratio.

Genetic relations of Ff and Aa to other genes studied

When tested for linkage, both the fasciated and the green-stemmed factors have given F_2 ratios fitting well to 9:3:3:1 in all combinations with

the genes of the first linked group and with those controlling foliage color (*Ll*), fruit flesh color (*Rr*), fruit skin color (*Yy*) and shape of leaf (*Cc*).

Linkage relations within the third group

Crossing the "Green Stem" mutant with its small, 2-celled, round Cherry type fruits, with pollen from a purple-stemmed linkage tester, bearing large, irregular fasciated fruits, brings together *aaFF* and *AAff* genotypes. The purple-stemmed F_1 hybrids bore bilocular, round fruits, whose selfed seed produced in the second hybrid generation: 80 purple stemmed and non-fasciated (mostly bilocular) (*AF*): 41 purple and fasciated (*Af*): 48 green and non-fasciated (*aF*): 1 green and fasciated (*af*). The repulsion between green stem (*a*) and fasciated (*f*) factors is as impressive in the field as it is obvious in the figures, and may be of some practical interest. By the coefficient of association ($Q = .913$) and ratio fitting the data is interpreted as showing about 14 percent crossing over, allowing an equal crossover frequency for both sexes.

In this as in the preceding instance of linkage I stress rather the establishment of the existence of the associations themselves, than any particular crossover values. Estimates from comparatively small F_2 repulsion groups are so liable to large error, due to the magnification of the chance fluctuations of the small double recessive class, that it is safer to resort to the easily-made backcrosses before assigning more precise values.

CONCLUSION

During the eight years that these linkage experiments have been in progress, over 35,000 plants have been grown and classified or studied in the various generations. Since chief attention was directed from the first on the presence of linkages and on linkage values quantitatively expressed, a considerable bulk of data has accumulated which, along with the pertinent facts from the literature, seem to lead to a few definite conclusions as to the organization of the germinal material in the tomato. An attempt has been made to state these conclusions conservatively and fairly in the resumé below, where are shown the genes determined in the linkage groups I, V and VI, and the "markers" assigned to groups II, III and IV, the group numbers being left the same as in the earlier work (MACARTHUR 1926, p. 401).

Until 3- and 4-point tests or suitable backcrosses are completed, no claim is made that the genes of group I are arranged in their final correct order, or properly spaced for mapping the chromosome, or that factors in

groups V and VI are more than roughly located; such an order and such unit distances afford the best interpretation of the data from all present sources.

I	II	III	IV	V	VI
0.0 dwarf	Yellow flesh	Clear skin	Potato leaf	0.0 fasciated	0.0 lutescent
3.4 peach				14.0 green stem	26.0 uniform (pale)
12.0 oval-pear					
32.0 compound					

SUMMARY

1. Adequate new linkage test have substantiated the earlier conclusion that in the tomato, linkage exists between factors differential for: *tall-dwarf* (*Dd*) stature, *smooth-peach* (*Pp*) skin, *spheric-elongate* (*pear* or *oval*) ($Oo=P_rp_r$) fruit shape, and *simple-compound* (*Ss*) inflorescence.

Within this *first linkage group* (group I) the linear order of genes, not yet determined by a 4-point test, is believed to be: *d-p--o*(p_r)------*s* (table 4, p. 415); if so the crossover values correspond closely to map distances up to nearly 12 units and fall short for greater distances, suggesting a chromosomal organization after the classical type (figure 1).

2. A new *linkage group* (group VI) contains two factor pairs, one (*Ll*, formerly called *Gg*) controlling green *versus* yellow ("lutescent") foliage color, and the other (*Uu*) regulating the distribution of chlorophyll color over immature fruits (see page 415).

Between *Ll* and *Uu* an F_2 repulsion ratio was obtained indicating probable linkage with about 26 percent crossing over. This still requires confirmation in backcrosses.

3. Another new *linkage group* (group V), also containing two factor pairs, is described. Genes (*Ff*) for non-fasciated *versus* fasciated fruit are closely linked (14 percent crossing over) with genes (*Aa*) controlling purple (with anthocyanin) *versus* green (without anthocyanin) stem.

4. The available linkage data is summarized on p. 419 showing the three established groups and the independent "markers" for three others.

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SEX-LINKED CHARACTERS OF POULTRY[1]

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INTRODUCTION

The sex-linked group of characters in poultry is of interest for several reasons. Some members of this group were not only among the first characters of the domestic fowl to be subjected to genetic study but were also among the first recognized examples of sex-linked inheritance. Barring and gold are two of the earliest recognized and best known characters of this group. Although a number of autosomal characters are well known, no linkage groups other than those whose genes are in the sex-chromosome are yet well established. The comparatively large number of sex-linked characters is also of interest since cytologists have shown that the sex-chromosome is by far the largest chromosome of the group characteristic of this species.

A more accurate knowledge of the linkage relations of characters of the sex-linked group is of interest in its relation to the genetics of this animal. The relations of this group of characters also have considerable practical interest since, through linkage it may be possible to handle, more effectively, the more or less intangible quantitative characters such as egg production and size.

METHODS AND MATERIAL

The study here presented was made during the period of 1923 to 1927 at the KANSAS STATE AGRICULTURAL COLLEGE. The material used was a part of the regular Experiment Station flock of this institution.

[1] Contribution number 43 from the Departement of Poultry Husbandry, KANSAS STATE AGRICULTURAL COLLEGE.

The breeds used in the study were the Single Comb White Leghorn, Jersey Black Giant, Silver Penciled Rock, Brown Leghorn, Buff Leghorn and Barred Plymouth Rock. By previous studies (HADLEY 1913) the Single Comb White Leghorn breed had been shown to carry the factor for barring and the writer (1924) has shown that this breed also carries the factor for rapid feathering. The Jersey Black Giant breed exhibits the extended black which is the allelomorph of barring and was found by the writer (1923) to possess the allelomorph of rapid feathering (slow feathering). This breed was also found to carry the determiner for dark shank. The Silver Penciled Rock, like most varieties of this breed, was found to carry the determiner for slow feathering and also to exhibit the silver color. The Brown Leghorn breed has been shown by GOODALE (1917) to carry gold and was found in this study to belong to the rapid feathering group. The Buff Leghorn was found to carry gold and rapid feathering while the Barred Plymouth Rock proved to be slow feathering and by previous studies (DUNN 1924) had been shown to carry the factor for silver. The symbol, B, has been used for barring, and b for its allelormorph; S for silver and s for its allelormorph; $S_l - s_l$ have been used for the pair determining rate of feathering, and $Y - y$ for the sex-linked shank color. For the breeds used, the genetic constitution with regard to the genes of the sex-chromosome may be symbolized as follows:

Single Comb White Leghorn	$BB\ s_l s_l\ YY\ SS$
Jersey Black Giant	$bb\ S_l S_l\ yy\ SS$
Silver Penciled Rock	$bb\ S_l S_l\ YY\ SS$
Brown Leghorn	$bb\ s_l s_l\ YY\ ss$
Buff Leghorn	$bb\ s_l s_l\ YY\ ss$
Barred Plymouth Rock	$BB\ S_l S_l\ YY\ SS$

In the earlier studies upon rate of feathering chicks were examined for the presence or absence of tail feathers at the end of the first, second, and third weeks. It was later found, however, that examinations made at ten days and three weeks were more reliable and in later studies records made at these ages were used for classification. All individuals were described at three different ages—one day, three months and six months. No attempt was made to classify chicks for shank color before the age of three months and descriptions made at this time checked very closely with the six-month description. Descriptions made at hatching were not found entirely reliable for classifying chicks for the gold and silver colors, nor for barring. So wherever possible the six-month description was depended upon for classification. Earlier descriptions were utilized in cases of death before maturity and were also used to check records made at the later

period. For all records chicks were identified only by wing-band number so that the experimenter's judgment would not be biased by a knowledge of the expected results.

FACTORS STUDIED

Four genes of the sex-chromosome were considered. Gene *S* and its allelomorph determine the presence of the gold or silver color of the plumage. Gene *B* together with its allelomorph determine the expression of barring of the feathers. Another gene of the sex-chromosome is S_l which, with its allelomorph, determines the age at which the chick down is replaced by feathers. The fourth gene studied is *Y* which affects the pigmentation of the shank.

The characters gold and silver have been found to be somewhat unsatisfactory ones with which to work since they are expressed only in regions where melanic pigment is absent. For plumage patterns where the black pigment has rather wide extension the classification of this character is sometimes difficult. In crosses of several breeds, the writer has observed the appearance of a yellowish to reddish brown color which usually appears upon the shoulders of males and breasts of females. In males this color is sometimes extended to the neck region. This color has been observed upon males which were known to be of the *Ss* composition and in some cases has led to confusion in classifying the phenotypes. The appearance of this color upon birds carrying *S* would sometimes lead to erroniously classifying them as homozygous golds.

The factor for barring has also been found difficult to study especially in cases where there are involved other pattern factors which restrict the extension of melanic pigment. This difficulty was encountered in the cross between the Barred Plymouth Rock and the Buff Leghorn since the latter carries the Columbian pattern which restricts the black pigment to the neck region and to the extremities of the tail and wings.

The gene for rate of feathering was found not to be interfered with by any other factors. All chicks not showing definite tail feathers upon the tenth-day examination were classed as slow-feathering. This character is fairly clean-cut in its expression and there is very little error in classification. A few exceptions were found in which birds known to be heterozygous for this factor, were rapid feathering. One such male when tested by breeding proved his heterozygous constitution. So although there is an occasional failure of the dominance of slow feathering this does not occur sufficiently frequently to cause any serious error in classification.

The gene which determines shank color is also very definite in its expression but is probably masked by certain plumage colors. The contrasted characters were yellow and blue shank. Although SEREBROVSKY considers the association of certain shank and plumage colors to be a matter of close association of their genes on the chromosome, the writer is of the opinion that the apparent linkage of yellow shank to barring and dark shank to black plumage is merely an instance of the extension to the shank of the effects of the factor determining the pigmentation of the plumage. The evidence supporting this belief will be discussed later in this paper. Because of their masking effects certain plumage color classes were eliminated in the shank color studies.

EXPERIMENTAL RESULTS

For study of the linkage relation of rate of feathering with shank color and barring, the cross of the Single Comb White Leghorn by the Jersey Black Giant was utilized. Although the relationships of three genes were followed in this cross, it could not be made a three point experiment because of the masking effects of some of the characters involved. White plumage color interfered with the expression of barring and its allelomorph black. Also, barring and its allelomorph are believed by the writer to mask the shank colors. Thus the white segregates were classified for linkage relations of rate of feathering to shank color, while the colored segregates were grouped for the study of the linkage of barring to rate of feathering. This selection of material for the two studies is allowable since it is based upon the segregation of an autosomal character which should in no way affect the ratios of sex-linked characters.

Linkage of rate of feathering and shank color

For determining the relation between these two factors four different F_1 males were used and the results were from three different breeding seasons. One male was used for two succeeding seasons. The four males were mated with 38 females. The females were pure bred Jersey Black Giants, pure White Leghorns, and segregates from the cross. It was only where the females carried both recessive factors that the male offspring could be included in the data. This accounts for the fact that the females in table 1 greatly outnumber the males. In this table are shown the data for linkage relations of rate of feathering to shank color, grouped according to the different males used.

Earlier work had shown slow feathering to be dominant to rapid and yellow shank to blue. Since the parental combinations were rapid feathering and yellow shank for the White Leghorn and slow feathering and blue

shank for the Jersey Black Giant the two crossover classes in table 1 are rapid-feathering blue shanks and slow-feathering yellow-shanks. It is seen that the numbers in the four groups are quite similar, there being

TABLE 1

Linkage relations of rate of feathering and shank color.

NUMBER OF F_1 MALE	FEMALES				MALES			
	Rapid yellow	Rapid blue	Slow yellow	Slow blue	Rapid yellow	Rapid blue	Slow yellow	Slow blue
887M (1924)	12	6	21	15				
(1925)	6	4	6	5		1	1	
935M	33	29	29	28				
911M	14	10	17	17				
957M	13	7	10	12	16	7	15	13
Total	78	56	83	77	16	8	16	13
Grand total both sexes	94	64	99	90				

Crossovers	163
Non-crossovers	184
Crossover percentage	47.0

some shortage in the one crossover group, rapid-blue. The calculated crossover percentage is 47. This closely approximates independent assortment and shows that these two genes are widely separated on the sex-chromosome.

Linkage of rate of feathering and barring

In this study were used the same four F_1 males which were mentioned in the previous experiment. The colored segregates were classified for rate of feathering and the barred and non-barred condition. The white segregates were not included in these data since the expression of the barring factor is masked by the white factor. In table 2 will be found four groups—rapid-barred, rapid-non-barred, slow-barred, and slow-non-barred. Since rapid-barred and slow-non-barred were the combinations found in the parental stocks, the other two classes constitute the crossover groups. Here again since only a few of the females mated to the males being tested were of the double recessive constitution, the number of male offspring included is limited. The percentage of crossing over for the total figures in this experiment is 48.6. This value approximates very closely that of

the previous experiment and is what would be expected from situations of independent assortment. Thus the genes for barring and shank are about equally distant from the gene for rate of feathering.

TABLE 2

Linkage relations of rate of feathering and barring.

NUMBER OF F_1 MALE	FEMALES				MALES			
	Rapid barred	Rapid non-barred	Slow barred	Slow non-barred	Rapid barred	Rapid non-barred	Slow barred	Slow non-barred
887M (1924)	9	16	8	16				
(1925)	1	4	2	4		2	1	3
935M	11	5	2	9				
911M	9	6	4	3				
957M	3	5	4	1	1	6	5	4
Total	33	36	20	33	1	8	6	7
Grand total both sexes	34	44	26	40				

Crossovers	70
Non-crossovers	74
Crossover percentage	48.6

Linkage of rate of feathering and gold

For this study it was necessary to make use of a cross differing from that used in the previous experiments. The F_1 males tested for percentage of crossing over were the offspring of a cross of a Brown Leghorn male by Silver Penciled Rock females. Two males were tested by mating to 17

TABLE 3

Linkage relations of rate of feathering and gold.

NUMBER OF F_1 MALES	FEMALES				MALES			
	Rapid gold	Rapid silver	Slow gold	Slow silver	Rapid gold	Rapid silver	Slow gold	Slow silver
987M	74	20	3	50	59	16	2	38
989M	82	8	4	67	68	24	5	56
Total	156	28	7	117	127	40	7	94
Grand total both sexes	283	68	14	211				

Crossovers	82
Non-crossovers	494
Crossover percentage	14.2

females all of which carried both recessive factors. Some of the females were sisters of the two males, other were F_1 females resulting from crosses of Brown Leghorn to Buff Leghorns and Rhode Islands Reds, and a third group were pure Brown Leghorns. Since they all carried gold and rapid feathering their other factors are of no interest here. In the Brown Leghorn breed rapid feathering was associated with gold and in the Silver Penciled Rock, silver was associated with slow feathering. Thus in table 3, of the four classes—rapid-gold, rapid-silver, slow-gold, slow-silver—the two middle ones include the crossover individuals. Table 3 shows that the original combinations tend to remain unbroken, there being 494 non-crossovers to 82 crossovers. The percentage of crossing over for the total of all crosses is 14.2.

As was earlier stated, the writer experienced some difficulty in distinguishing the gold and silver genotypes. This was particularly true of the male segregates since many had the duck-wing pattern in which the black areas limit the expression of gold and silver to the neck and shoulder regions. There also appeared to be involved an additional reddening or bronzing factor which when present in silver individuals made them difficult to separate from the lighter golds. In the female the gold and silver were less restricted by black and little difficulty was experienced. Since the ratio of gold and silver males is about equal and closely approximates that of the females, it is believed that the errors of classification were not large.

EFFECT OF AGE OF MALE UPON CROSSING OVER FREQUENCY

Since in the data for linkage relations of rate of feathering to shank color and barring, one male was used for two succeeding years, there is opportunity to consider the effect of age of the male upon the crossover percentages. Male 885M had 54 offspring during his first breeding year and 21 during the second, from which the crossover percentages between genes S_l and Y could be calculated. During the same years he had 49 and 11 offspring from which the linkage relations of S_l and B were calculated. For his first breeding year this male gave a 50 percent crossover value and for his second year a value of 47.6 percent between S_l and Y. For the linkage relations between S_l and B he gave a crossover value of 49 and 54.5 percent for the same two years. The numbers involved here are too small to base any reliable conclusions, but since the results of the two crosses are conflicting, it cannot be said that there is any evidence that the difference in age between the first and second breeding year of a male influences the crossover frequency.

DOUBLE CROSSING OVER

One cross was made which involved three sex-linked factors. The interference of these factors, one with another, was not so great but that some estimate of the degree of double crossing over could be made. This was a cross of the Buff Leghorn and Barred Plymouth Rock breeds. The former was of the composition $s_l\ s\ b$ and the latter $S_l\ S\ B$. The shortage of certain classes indicates that there were errors of classification in these data, but since the single crossover values, excepting those for S_l and S, are similar to those from other experiments, the results are here presented. The shortage of crossover between S_l and S will also reduce the double crossovers since these points are involved in double crossing over.

Non-crossover	65
Single crossover between S_l and B	39
Single crossover between S_l and S	4
Double crossover involving the above two	4

The above results are based upon the assumption that the genes are in the order S_l-S-B. The evidence, although not entirely conclusive, points to this arrangement. If the arrangement is S-S_l-B it would mean only a slight change in the last two figures. The foregoing results may be taken to indicate that double crossing over takes place relatively frequently in the sex-chromosome. With the same genes involved, SEREBROVSKY (1927) also found evidence of double crossing over in the sex-chromosome.

DISTRIBUTION OF FACTORS ON THE SEX-CHROMOSOME

With our present knowledge of the linkage relations of the factors upon the sex-chromosome of the chicken, it is of interest to map tentatively the genes of this chromosome. In addition to the relationships presented in this paper other workers have published data for the linkage relations between barring and gold. HALDANE (1921) from a limited amount of data (78 birds) first determined the percentage of crossing over to be 34.6. AGAR (1924) with a total of 56 birds, placed the crossover percentage at 46.4 for one cross and at 35.7 in another cross involving 28 individuals. SEREBROVSKY (1927) with a total of 77 individuals found the crossover value to be 43 percent. Most of the workers mention the difficulty of classification in studies of these factors. From the cross of Barred Plymouth Rock by Buff Leghorn the writer obtained a ratio 86 non-crossovers to 72 crossovers giving a crossover value of 45.6 percent. Due to the interference of the Columbian pattern with barring and barring with gold, it is felt that the ratio given is not entirely reliable.

However, the results of AGAR, SEREBROVSKY and the writer check very closely, 46.4, 43 and 45.6 percent. Each of the three above workers used a different breed as the carrier of gold.

In 1924 the writer gave the crossover value between rate of feathering and barring to be 47.1. Additional data slightly increased this value to 48.6 percent. SEREBROVSKY (1927) obtained a somewhat lower value, 44 percent.

The data available in 1924 led the writer to assign a crossover value of 48 percent from rate of feathering and shank color. Including additional data, the value given in this study is 47 percent. SEREBROVSKY (1927) gave for this same pair of factors a value of 49 percent.

SEREBROVSKY (1927) found the crossover value for gold and rate of feathering to be 19 percent. From a much larger number of birds the writer calculated the value to be 14.1 percent.

SEREBROVSKY (1927) has also attempted to determine the linkage relations of the shank color and barring pair of factors. He found two questionable crossovers among 69 individuals. He held that the genes from these two characters were quite closely associated. The writer's own data, showing these two factors to be about equally distant from the rate of feathering gene, would substantiate the suggestion that they are in the same general region of the sex-chromosome. It is doubted, however, that it is possible to determine the linkage relation of these two genes because of the interference of their characters. It is the opinion of the writer that what SEREBROVSKY considered as very close linkage is a situation of extension to the shanks of the effects of factors determining plumage pigmentation. The appearance of only dark-shanked black birds is due to the extension of the black pigment to the shanks as well as the feathers. The barring factor is held to be a partial inhibitor of black plumage pigment. This same factor might also inhibit black pigment of the shanks. It is true that most barred birds do show some black pigment on the shanks. However, all barred breeds of poultry have shanks which are predominately yellow. It might be held that the explanation for this lies in the existence of close linkage between barring and shank color. If barred breeds are yellow-shanked because of close linkage between shank color and barring, then barred breeds crossed to yellow-shanked breeds should produce only yellow shanks. Individuals of this breed are known to carry the factor for black plumage so if the yellow shanks are due to the inhibitive effect of the barring factor, then segregates released from the restrictions of this factor should have black shanks. A cross of

a Dark Brahma male by Barred Plymouth Rock females produced barred yellow-shanked sons and black plumage, black-shanked daughters. The Dark Brahma breed exhibits the silver duck-wing pattern in the male and the silver tri-penciled pattern in the female. The daughters of this cross would not carry the barring factor since they receive their only sex-chromosome from the father, who himself did not have this factor. Since the females here when released from the inhibiting effects of the barring factor are not only black color but black-shanked would seem to support the view that barred birds are yellow-shanked because of the inhibiting action of the barring factor, or more particularly that the extension factor influences shank color. The only difference between the sons and daughters of this cross is that the former carry the barring factor (and other sex-linked factors of the father) while the latter do not.

There are some black breeds (Black Leghorn) which have shanks that are predominately yellow. Individuals of the Black Leghorn breed, however, frequently show considerable black pigment on the shanks so the reduction of black here may be due to some other factor.

The sex-linked allelomorph of yellow shanks, which is blue, has been shown by BARROWS (1914) to be of this color because most of the pigment is dermal in location. The black pigment as seen through the epidermal layers appears dark blue. The shanks of black plumaged segregates are black and not blue. This is because birds of this color have the epidermal layer heavily pigmented. This being true, it can readily be seen that the pigmentation of the shank due to the extensive factor affecting black in the plumage would mask the sex-linked factor, the expression of which is largely in the dermal tissue. For the reasons given above, crosses involving both *Y* and *B* and their allelomorphs cannot be used for direct calculation of the map distance between these two genes.

SEREBROVSKY and WASSINA (1927) have suggested the probable arrangement upon the sex-chromosome of the four genes considered. They appreciated, as does the writer, that linkages as loose as those found cannot be used for accurate estimations of map distance. However, since we have information upon four different genes, and since the data presented by the various workers are in fair agreement, it is considered worth while to estimate roughly the positions which the accumulated data will indicate that these genes occupy.

Figure 1 shows the linkage relations determined by the various workers for the four factors considered. All studies have shown a high percentage of crossing over between rate of feathering and both barring and shank color. This would indicate that it is widely separated from these two fact-

ors and since its crossover value with each is about the same, these two factors are probably closely associated. Just how closely barring and shank color are associated, linkage as loose as those involved will

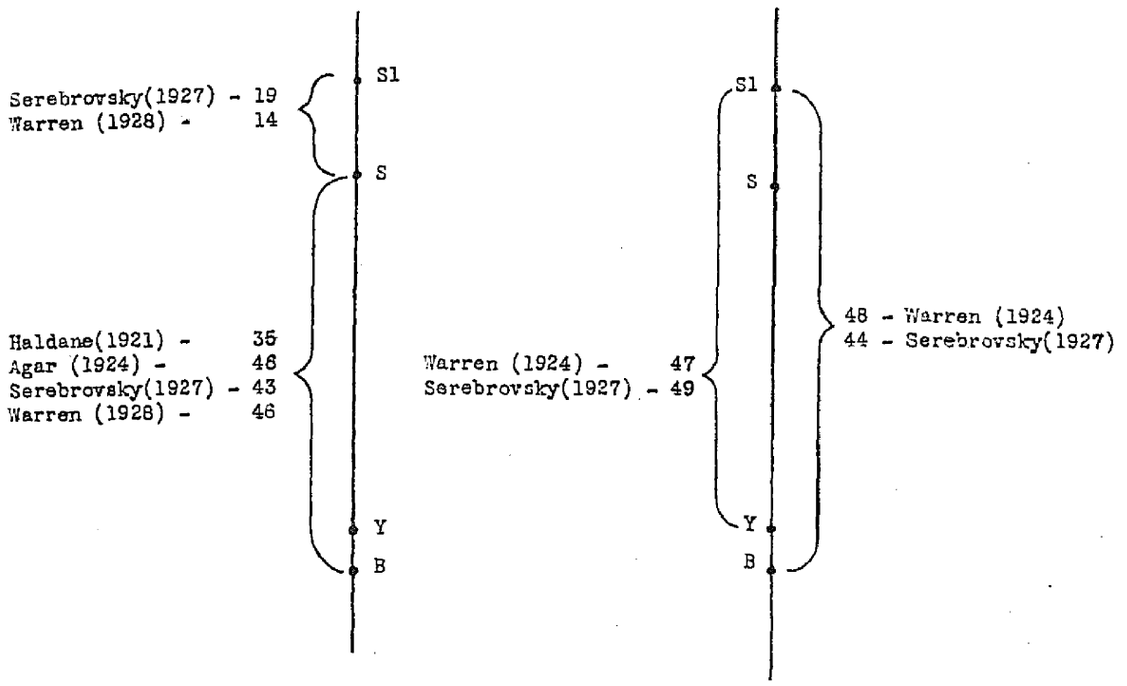


FIGURE 1.—Showing a tentative arrangement of the known genes of the sex-chromosome based upon crossing over percentages calculated by the various workers.

not indicate. Interference of characters will not permit a direct calculation of their crossover values. Thus the relative positions of barring and shank color with respect to rate of feathering cannot be reliably predicted and the tentative positions indicated for these two factors may quite possibly be reversed.

Since all data have shown that gold exhibits very loose linkage relations with barring, these two genes must be distantly separated. Since the crossover value ($14\pm$) between gold and rate of feathering is much lower than the value between it and barring ($45\pm$), gold and rate of feathering must be in the same end of the chromosome. Although the data are not entirely conclusive, gold has been tentatively placed between rate of feathering and barring. The data of the writer, which includes a larger number of individuals than those of all other workers combined, confirm the arrangement of genes suggested by SEREBROVSKY (1927). It can at least be said that genes *B* and *Y* are associated in one section of the chromosome and that genes S_l and *S* are about 14 units apart in a distant section of the same chromosome.

The linkage relations of gold to shank color have not yet been determined by anyone and the writer is not familiar with any pure breeds whose constitution is such as to make them suitable for determing these relations.

No other sex-linked characters have definitely been determined in poultry. PUNNETT (1923) mentions the black skin pigment in the Silky breed as exhibiting sex-linked behavior but suggests that it may be due to the action of the gene *V*. LEFEVRE and RUCKER (1923) have listed the spangling pattern as belonging to this group, but other workers have not been able to substantiate their conclusion. So until other workable sex-linked factors of the fowl have been found the chromosome cannot be definitely mapped.

Probable errors have not been calculated for the crossover values given in the foregoing tables since it is believed that the error of map distance due to double crossing over is probably greater than that of random sampling. The error due to double drossing over cannot be estimated until intervening genes have been found.

SUMMARY

1. The crossover value between rate of feathering and shank color was found to be 47 percent.
2. Rate of feathering and barring gave a crossover value of 48 percent.
3. The factors for rate of feathering and gold gave a crossover value of 14 percent.
4. Gold and barring gave a crossover value of 45 percent.
5. The crossover value for the factors barring and shank color cannot be directly calculated because of masking effects.
6. Accurate mapping of the genes on the chromosome cannot be accomplished until more closely associated points are found.

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TRANSPARENCY AND MOTTLING, A CASE OF MENDELIAN INHERITANCE IN THE GOLDFISH *CARASSIUS AURATUS**

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INTRODUCTION

In the spring of 1924 I made a preliminary study of the heredity of goldfish by crossing various breeds of goldfish and mating domesticated breeds of goldfish with the wild goldfish. During the progress of this preliminary study I noticed that the inheritance of one of the characters under investigation was rather simple.

This character was called "transparent scale" in my previous paper (CHEN 1925). The goldfishes of this breed have only a few normal scales, the remainder of the body being apparently naked. The apparently naked part of the body is really covered by scales which, on account of the lack of a layer of reflecting tissue on their inner side, are as transparent as glass. This breed of goldfish is called "shubunkin" in Japan (MATRUBARA 1908) and is known as "calico" in the United States (INNES 1917).

Beginning in the spring of 1925 I started more extensive experiments to investigate the mode of inheritance of this character, "transparent scale"or "calico." In the summer of 1925 I obtained enough evidence to prove that the inheritance of this character is Mendelian. A preliminary report of this discovery had been made (CHEN 1926). The present paper is the final report of this investigation.

* The GALTON AND MENDEL MEMORIAL FUND contributes the accompanying colored illustrations.

MATERIAL AND METHODS

The materials of this investigation were the transparent scaled fancy goldfishes, the normal scaled fancy goldfishes, and the normal scaled wild goldfishes. The fancy goldfishes were obtained from a local goldfish breeder, the wild ones being obtained from the food-fish market of Nanking.

The fishes were bred and grown in water reservoirs made of burned clay. These reservoirs were made in the shape of a huge cup with the upper diameter about 38 inches, lower diameter about 19 inches, and a depth of about 32 inches. Such a reservoir is large enough to contain about eighty young fishes to a size capable of reproduction in the next breeding season.

Goldfish take one year for a generation. The breeding season of the goldfish at Nanking begins in the middle of April and ends in the middle of June, lasting about two months. During this period a single male fish may mate four or five times and a female fish two or three times with an interval of about ten days between successive matings.

I tried to make the different crosses of goldfish by means of artificial fertilization. These attempts failed. Hence all the various crosses in my experiments were made by the ordinary method of natural fertilization as employed by the common goldfish breeders except with some necessary precautions.

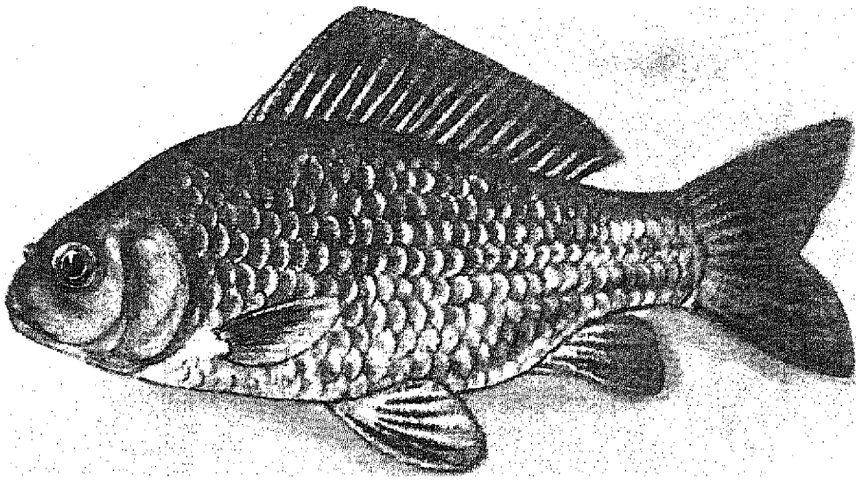
At the beginning of the breeding season I separated the female and male fishes into different reservoirs. The male fish is distinguished from the female by the presence of numerous small white tubercles, called the "pearl organs," along the front rays of the pectoral fins and on the external side of the two opercula. This separation of the females from the males was needed in order to avoid the loss of the eggs through the free matings in a reservoir as soon as the breeding season began and thus to insure the females to have eggs to lay when these were required for the experiments.

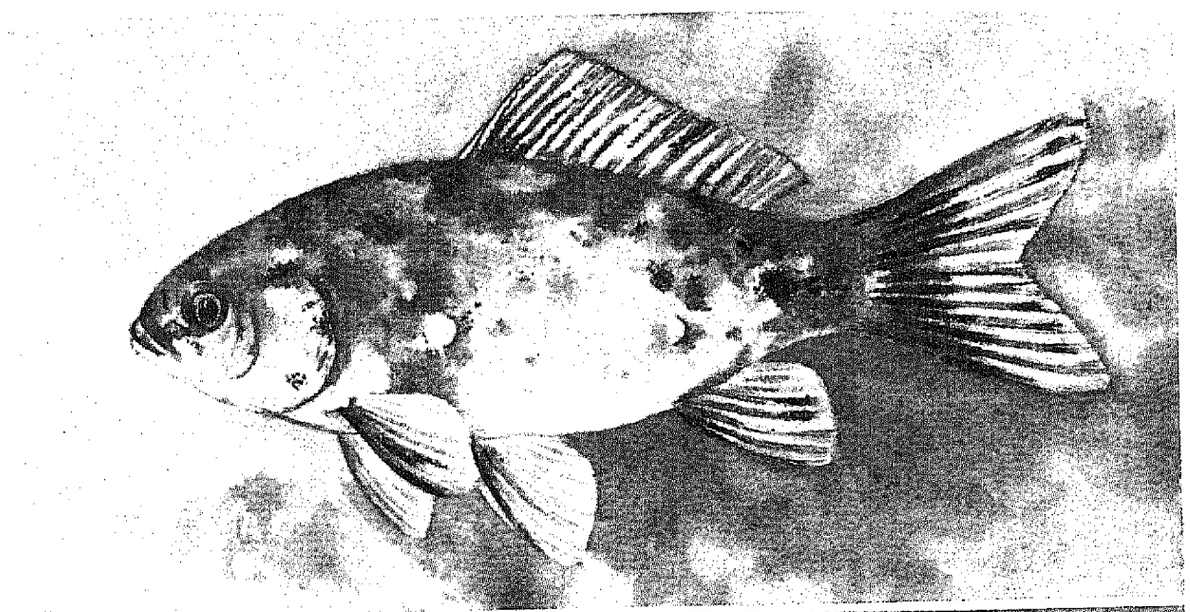
In mating goldfish the practice of the common breeders is to mate two or three female goldfishes with three or four males. Since such a practice would introduce some errors into the experimemts, I always mated one female with one male in a reservoir.

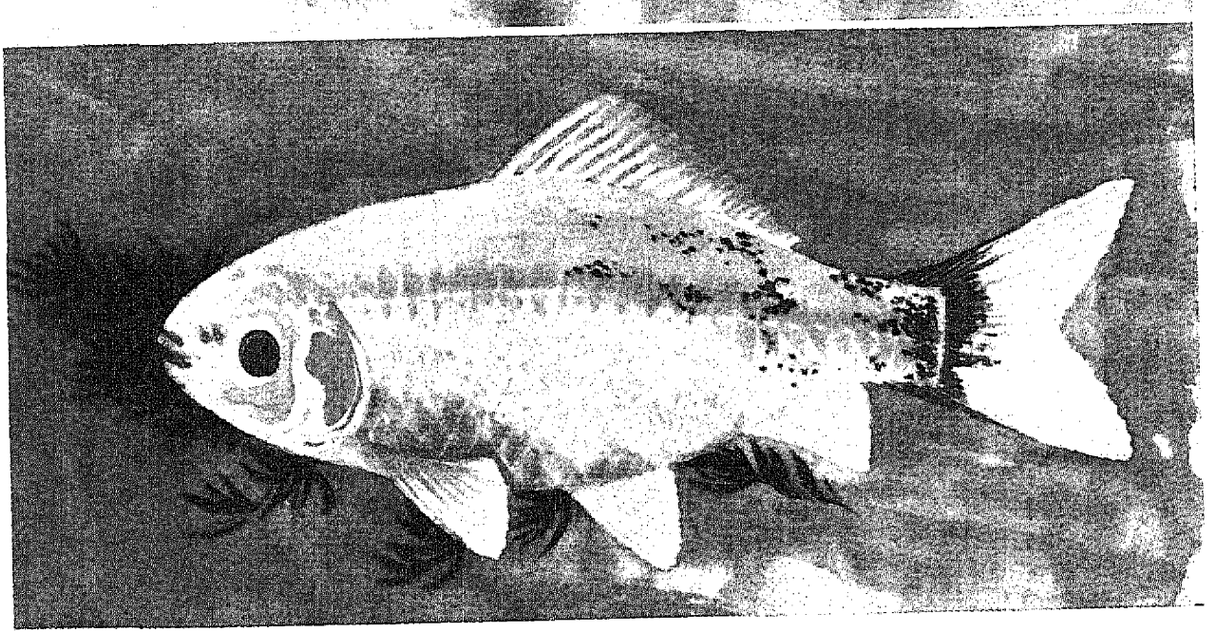
During mating the eggs of the goldfish were deposited on a bunch on water plant (Myriophyllum) which had been introduced into every mating reservoir. The eggs hatch from three days to a week after fertilization depending upon the temperature of the water in the reservoir.

LEGEND FOR PLATE 1

FIGURE 1.—Normal scaled fish.
FIGURE 2.—Heterozygous mottled fish.
FIGURE 3.—Homozygous transparent fish.







About two days after hatching, when the young fish were just able to swim freely, they were fed with finely pulverized yolk of boiled chick eggs. About three days later I began to feed the young fish with living cyclops and about two weeks after hatching the young fishes were fed with Daphnia. The goldfishes grew very rapidly on this diet.

About forty days after hatching the young fish had grown to about 25 mm in body length, being a size large enough to be examined with the naked eye. At this time the character of transparent scale had developed to such an extent that the fish with this character might be distinguished from the normal scaled fishes. The data concerning the offspring of the matings were mostly taken at this time. Sometimes the progeny of a single mating amount to about seven or eight hundred.

Frequently the young offspring were discarded after being examined, classified, and recorded. Sometimes these fishes were reserved for further examination. The young fishes at this time, about forty days after hatching, varied greatly in size. Goldfishes are cannibals and if the small fishes remain with the larger ones, the latter will devour the former. In a heterogeneous group of genotypically different classes of fish the quick growing will soon devour the slow growing and the former will survive while the latter perish. In order to avoid the introduction of this source of error into my experiments, I always separated the offspring of a single mating, at the age of about forty days after hatching, into two groups according to the size of the young fishes and reared these two groups separately in different reservoirs. Thus the slow-growing class had as good a chance to survive as the quick-growing class.

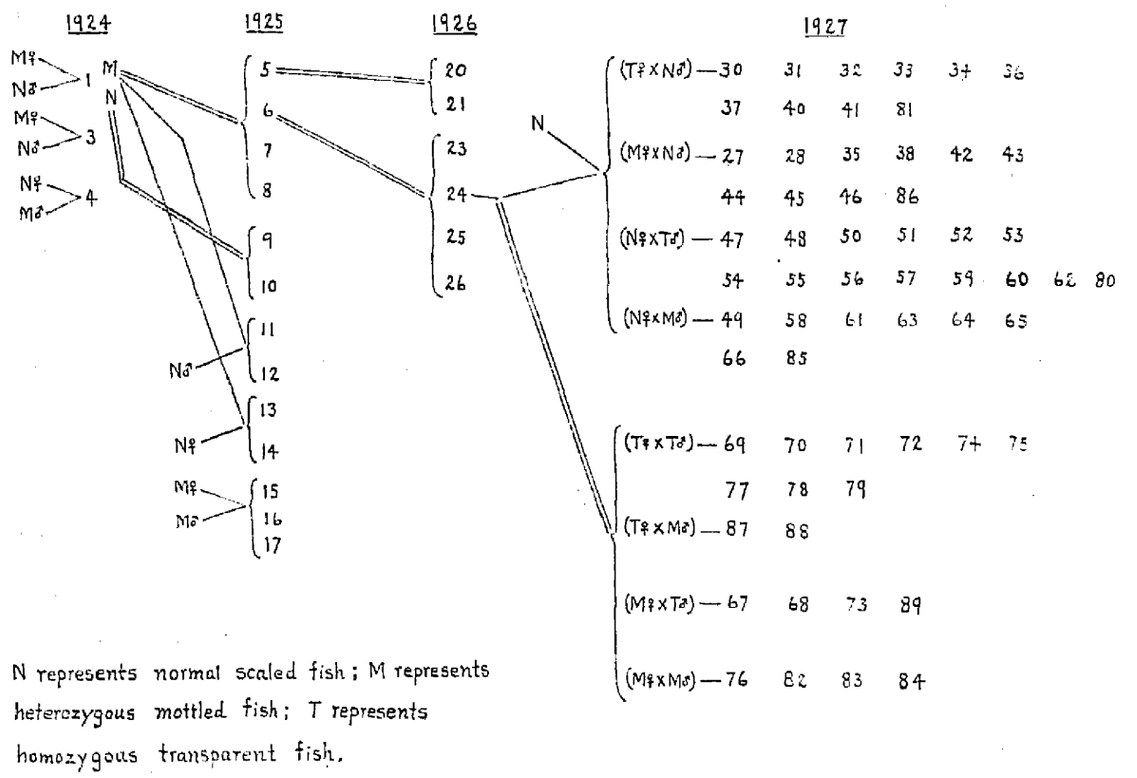
NARRATIVE OF THE EXPERIMENTS

1924. The parents and the F_1—In the spring of 1924 I made the following crosses (figure 1):

1. Transparent scaled fancy goldfish ♀ Normal scaled wild goldfish ♂
2. Normal scaled wild goldfish ♀ Transparent scaled fancy goldfish ♂
3. Transparent scaled fancy goldfish ♀ Normal scaled fancy goldfish ♂
4. Normal scaled fancy goldfish ♀ Transparent scaled fancy goldfish ♂

With the exception of the mating number 2, all of the matings produced offspring. The F_1 fishes of all the fertile matings were unexpectedly of two classes. One class of the offspring had transparent scales and mottled condition while the other class had normal scales. The normal scaled offspring of family 1 were exactly like the wild goldfish (plate 1). The transparent-scaled offspring of the same family were similar to the wild goldfish in every respect except with transparent scales and the mottled

pattern (plate 1, figure 2). The latter class of fish with a long body and unpaired fins resembles closely a Japanese breed of goldfish called "shubunkin" but is an entirely new form to the goldfish fanciers in China. I found both females and males among the transparently scaled and the normally scaled classes.



The two classes of offspring were of approximately equal numbers as shown by the data in table 5. This result was interpreted by assuming that the transparent scaled parents of the families 1, 3, and 4 were all heterozygous with respect to a pair of allelomorphic factors one of which when homozygous was responsible for the production of the transparent scales while the other factor, when homozygous, would cause the production of the normal scales. All the normal scaled parents were homozygous for normal scales. The gene for the production of the transparent scales is accordingly dominant to the gene for the production of the normal scales. Hence the transparent scales and the mottled pattern appeared on the body of the heterozygous fish and the offspring of the three families were of the two classes, heterozygous mottled fish and homozygous normal scaled fish.

1925. The F_2 — From the offspring of family 1 I made four matings between the transparent scaled females and the similarly scaled males. The offspring of these matings were, as expected, of two classes, transparent

scaled and normal scaled. The number of individuals in these two classes and their ratios are presented in table 3, families 5, 6, 7, and 8.

The data in table 3 follow closely the typical Mendelian ratio of the F_2 generation, three to one. The assumption that the original parents of 1924 were heterozygous and all the transparent scaled offspring were also heterozygous was thus confirmed.

In addition to the four matings mentioned above, I had also made four backcrosses between the transparent scaled offspring of family 1 and the normal scaled fancy goldfishes. The results of these matings are presented in table 5, families 11, 12, 13 and 14. The data of these back crosses also prove that the transparent scaled offspring were heterozygous as expected.

The matings between the normal scaled females and males of the offspring of family 1 had also been made. One of these matings produced 761 normal scaled offspring (family 9). Another mating produced 248 normal scaled offspring (family 10). Neither of these matings produced a single transparent scaled fish. This result is in accord with the assumption that the original normal scaled parents and the normal scaled offspring were homozygous and that the normal scaled condition is recessive.

1926 The F_3 generation—On account of a necessary absence from Nanking during the breeding season of 1926 I did not have time to make more extensive experiments than nine matings between the transparently scaled females and males of the F_2 generation.

Only six out of the nine matings produced offspring forming the families 20, 21, 23, 24, 25, and 26. Families 20, 21, 23, 25, and 26 consisted of transparent scaled and normal scaled offspring in the typical Mendelian ratio of three to one (table 3). Family 24, however, consisted of 346 transparent scaled fishes together with only one normal scaled fish. The exceptional normal scaled fish was thought to have been accidently introduced into this family from some other source due to the carelessness of the aquarium keeper during my absence from Nanking.

1927 The F_4 generation.—In the spring of 1927 I took twenty females and twenty males from the offspring of family 24 and backcrossed them with normal scaled goldfishes. I obtained the following results:

Nine transparent scaled females and thirteen transparent scaled males in mating with the normal scaled goldfish produced only transparent scaled offsprings. These twenty-two transparent scaled fishes were thus proved to be homozygous with respect to the gene for the transparent scale.

Nine transparent scaled females and seven transparent scaled males in mating with the normal scaled goldfishes produced offspring of two kinds in approximately equal numbers. These sixteen transparent scaled fishes were thus proved to be heterozygous, with one gene for transparent scale and one gene for normal scale.

Two transparent scaled females in the backcross with normal scaled goldfishes failed to produce offspring.

By the above mentioned breeding work I proved twenty-two transparent scaled fishes to be homozygous and sixteen transparent scaled fishes to be heterozygous. These two classes of fishes were examined in detail. I found that they have distinguishing features which although difficult to define in terms of words, are, on the whole, easily recognized as soon as it has once been noted. Figure 2 of plate 1 shows a heterozygous transparent scaled fish. Figure 3 of the same plate shows a homozygous transparent scaled fish. Detailed descriptions will be made later.

After I ascertained the genotypes of the thirty-eight transparent scaled fishes I made the following matings between the females and the males of these thirty-eight fishes near the end of the breeding season:

1. Nine matings between the females and the males of the homozygous transparent scaled fishes.
2. Two matings between the homozygous transparent scaled females and the heterozygous males.
3. Four matings between the heterozygous females and the homozygous transparent scaled males.
4. Four matings between the females and the males of the heterozygous fishes.

The above matings were made for the double purpose of testing whether the earlier assumptions were true and to see whether the ratios of the number of homozygous transparent scaled fishes to that of the heterozygous fishes were approximate to the expected ratios.

The results of these matings are presented in tables 1, 4, and 6. The data in these tables show that observed ratios are in close accordance with expectation.

TERMINOLOGY AND SYMBOLS

At the beginning of the present investigation I could distinguish only one kind of transparent scaled goldfish. This breed of goldfish is called "shubunkin" in Japan and "calico" in the United States.

After four years of experiments I found that the transparent scaled fishes could be separated into two classes, one of which is nearly completely

transparent while the other class always has a few normal scales and the characteristic mottled pattern. The former class is homozygous and the latter class heterozygous.

Since the homozygous transparent scaled fishes are phenotypically distinguishable from the heterozygous transparent scaled fishes, hereafter I shall call the fishes of the former class "transparent fish" and the fishes of the latter class "mottled fish."

The distinction between heterozygous mottled and normal fish is very sharp but the distinction between heterozygous and homozygous transparent fish is not so sharp and they even overlap when the fishes are young. According to the terminology of T. H. MORGAN (1919) a character is said to be recessive if the individuals with this character are sharply separable from the heterozygous individuals; a character is said to be dominant if the individuals with this character are overlapping with the heterozygous individuals. In this sense we may say that the "transparency" is a dominant character while the normal condition is recessive.

According to the methodology of T. H. MORGAN the symbol of the gene causing a dominant mutant character, like the bar eye of Drosophila, is a capital letter primed while the symbol to represent the gene causing the normal recessive character is the capital letter alone. Adopting this method of MORGAN I shall use the letter T to represent the gene causing the development of normal scales and the symbol T' to represent the gene causing the development of transparent scales.

DATA FROM BREEDING EXPERIMENTS

Normal scaled fishes breed true.—During the period of 1924 to 1927 I made numerous matings between the females and males of the normal scaled fancy goldfishes as well as the wild goldfishes. With the exception of a very few instances to be cited in a later section of this paper the offspring of all these matings consist of only normal scaled fishes. Furthermore I made two matings (families 9 and 10) of normal scaled fishes produced from a cross (family 1) between a transparent scaled fish and a normal scaled fish. One of the matings (family 9) produced 761 normal scaled fishes. The other mating (family 10) produced 248 normal scaled fishes. Both of these two matings did not produce a single fish with transparent scales.

Transparent fishes breed true.—In the spring of 1927 I made nine matings between the female and male transparent fishes. These nine matings produced many thousands of offspring, all of them were transparent fishes. The data are in table 1.

TABLE 1

Families which illustrate the true-breeding of transparency.

PEDIGREE NUMBERS			NUMBER OF OFFSPRING, ALL TRANSPARENT
Parents		Family	
♀	♂		
24(B5)*	24(C5)	69	81
(B6)	(C6)	70	183
(B7)	(C7)	71	41
(B8)	(C8)	72	122
(B10)	(C10)	74	150+
(B11)	(C11)	75	150+
(B4)	(C1)	77	100+
(B5)	(C2)	78	100+
(B6)	(C4)	79	100+
Total			1027+

* The number outside of bracket represents family number; those within the brackets represent individual numbers.

Transparent fish crossed with normal fish produce mottled fish.—In the spring of 1927 I made ten crosses between transparent females and normal males and fourteen crosses between normal females and transparent males. All these matings produced only fishes with the characteristic mottled pattern. The data of these matings are in table 2.

TABLE 2

Families which show the mottled heterozygous type in the F_1 of crosses between transparent and normal.

PEDIGREE NUMBERS		NUMBER OF OFFSPRING ALL MOTTLED	PEDIGREE NUMBERS		NUMBER OF OFFSPRING, ALL MOTTLED
♀ Parent*	Family		♂ Parent**	Family	
24(B4)	30	150+	24(C1)	47	350+
(B5)	31	174	(C2)	48	100+
(B6)	32	150+	(C4)	50	300+
(B7)	33	14	(C5)	51	300+
(B8)	34	100+	(C6)	52	200+
(B10)	36	80+	(C7)	53	100+
(B11)	37	150+	(C8)	54	100+
(B14)	40	100+	(C9)	55	300+
(B15)	41	14	(C10)	56	100+
(B10)	81	100+	(C11)	57	200+
			(C13)	59	200+
Total		1032+	(C14)	60	200+
			(C16)	62	200+
			(C5)	80	100+
			Total		2750+

* ♂ parents were normal scaled.

** ♀ parents were normal scaled.

Mottled fish never breed true, but always produce some normal fish and some transparent fish.—In the spring of 1925 I made seven matings between female and male mottled fishes. In the spring of 1926 I made five similar matings. The data of these matings are in table 3. These data show

TABLE 3

Segregations following mating mottled females with mottled males.

FAMILY NUMBER	TRANSPARENT AND MOTTLED OFFSPRING		NORMAL SCALED OFFSPRING		TOTAL NUMBER OF OFFSPRING
	Number	Ratio	Number	Ratio	
5	532	2.86	212	1.14	744
6	856	3.01	280	.99	1136
7	508	2.87	201	1.14	709
8	744	2.87	291	1.12	1035
15	482	3.08	143	.92	625
17	119	3.17	31	.83	150
20	236	2.84	96	1.16	332
21	181	3.07	55	.93	236
23	152	2.81	64	1.19	216
25	375	2.98	128	1.02	503
26	301	3.17	79	.83	380
Totals	4486	2.958	1580	1.042	6066
P.E.		±0.015		±0.015	

that among the offspring there were always produced some normal fishes. The ratios of the transparent and mottled fishes to the normal scaled fishes were all approximate to 3 to 1. The deviation of the observed ratios

TABLE 4

Segregations following mating mottled females with mottled males.

PEDIGREE NUMBERS			NORMAL SCALED OFFSPRINGS		MOTTLED OFFSPRING		TRANSPARENT OFFSPRING		TOTAL NUMBER OF OFF-SPRING
Parents ♀	♂	Family	Number	Ratio	Number	Ratio	Number	Ratio	
24(B12)	24(C12)	76	71	.83	170	1.98	102	1.19	343
(B2)	(C3)	82	110	.82	274	2.18	120	.95	504
(B9)	(C12)	83	82	1.27	117	1.80	60	.93	259
(B12)	(C15)	84	82	1.00	168	2.05	78	.95	328
Totals			345	0.96	729	2.03	360	1.00	1434
P.E.				±0.03		±0.036		±0.03	

from this expectation is 0.042 while the probable error of the expectation is 0.015. Since the deviation is less than three times the probable error the fitness of the observed ratio to the expectation is reasonably close.

In the spring of 1927 I made four additional matings between female and male mottled fishes. The results of these matings are in table 4 which show that among the offspring there were always about one fourth of transparent fish, one half of mottled fish, and one fourth of normal fish. The observed ratio is very close to the expectation. In the case of the total normal to the total offspring the observation is in perfect agreement with the expectation. The deviations of the other two ratios are only about once times the probable error.

Mottled fish crossed with normal fish.—I made fourteen matings of mottled females crossed with normal males and eleven matings of normal females crossed with mottled males during the breeding seasons of 1924 to 1927. Among these 25 matings I obtained quantitative data in eleven of them. The data are presented in table 5. I had also made a rough exami-

TABLE 5

Mottled heterozygotes backcrossed to the normal.

FAMILY NUMBERS	TYPE OF CROSS	MOTTLED OFFSPRING		NORMAL SCALED OFFSPRING		TOTAL NUMBER OF OFFSPRING
		Number	Ratio	Number	Ratio	
1	M♀×N♂*	156	1.08	133	.92	289
3	M♀×N♂	33	1.12	26	.88	59
11	M♀×N♂	654	1.05	592	.95	1246
12	M♀×N♂	557	1.04	511	.96	1068
43	M♀×N♂	127	1.06	112	.94	239
86	M♀×N♂	23	.79	35	1.21	58
4	N♀×M♂	120	1.05	111	.95	231
13	N♀×M♂	146	.92	171	1.08	317
14	N♀×M♂	573	.99	580	1.01	1153
64	N♀×M♂	101	1.06	90	.94	191
85	N♀×M♂	62	1.02	59	.98	121
Totals		2552	1.03	2420	.97	4972
P.E.			±0.01		±0.01	

* N represents normal scaled fish; M represents heterozygous mottled fish.

nation of the results of the 14 remaining matings and found them to consist of about one half of mottled fishes and one half of normal scaled fishes.

The data in table 5 show that all the matings produced about one half of mottled fish and one half of normal fish. The observed ratio fits very

closely to the expectation since the probable error of the expectation is ± 0.01 and the deviation of the observed ratio from the expectation is 0.03, being only three times the probable error.

Mottled fish crossed with transparent fish.—In the spring of 1927 I made two matings between transparent female and mottled male fishes and four matings between mottled female and transparent male fishes. The results of these matings are in table 6 which show that all these matings produced

TABLE 6

Mottled heterozygotes backcrossed to the transparent.

PEDIGREE NUMBERS			TYPE OF CROSS	MOTTLED OFFSPRING		TRANSPARENT OFFSPRING		TOTAL NUMBER OF OFFSPRING
Parents ♀	Parents ♂	Family		Number	Ratio	Number	Ratio	
24(B7)	24(C18)	87	T♀×M♂*	123	1.00	124	1.00	247
(B11)	(C19)	88	T♀×M♂	145	1.07	127	.93	272
(B1)	(C1)	67	M♀×T♂	24	.89	30	1.11	54
(B2)	(C2)	68	M♀×T♂	28	1.06	25	.94	53
(B19)	(C9)	89	M♀×T♂	138	.85	188	1.15	326
Totals				458	.96	494	1.04	952
P.E.					±0.02		±0.02	

* T represents homozygous transparent fish.

about one half of mottled fish and one half of transparent fish. The observed ratio fits very closely to the expectation since the deviation is only two times the probable error in the total offspring.

Besides the above observations families 24 and 73 had been roughly examined and found to consist of about one half of transparent fishes and one half of mottled fishes.

The character is not sex-linked.—During the progress of the breeding work I always paid attention to the problem of whether the character is sex-linked or not. Since the secondary sexual character, that is, pearl penis organ, of the male fish, is not visible before the fish is grown to considerable size I often dissected many young fishes to ascertain their sex. In all the various matings which I made I found that there were always female and male offspring in approximately equal numbers. Based upon all the facts which I observed in the breeding experiments I conclude that this character is not sex-linked no matter whether the inheritance of sex in fish be XY type, WZ type, or the modified XY type with genes on the Y chromosome as well as on the X chromosome as found in the case of *Aplocheilus latipes* by AIDA (1921).

THE MANIFOLD EFFECTS

I have already mentioned that by means of the progeny tests I had definitely found 22 transparent scaled fishes to be homozygous and 16 transparent scaled fishes to be heterozygous. With the two classes of transparent scaled fishes and the normal scaled fishes at my disposal I made a detailed examination of the various effects of the different combinations of the allelomorphic genes T and T'. This study is extended and confirmed by the examination of the offspring produced by the matings of the parents whose genotypic constitutions are definitely known.

Homozygous Normal.—With the presence of the gene T in duplex condition the fish has one or several layers of reflecting tissue covering the entire body including the head, trunk, and iris and excluding the pupils and the fins, being more abundant on the ventral surface of the body. The fish has also two kinds of chromatophores, that is, the black melanophores and the yellow xanthophores, distributed on the surface of the body and the fins and usually more abundant on the dorsal surface than on the lateral surface while on the ventral surface the chromatophores are generally absent.

With the exception of the breeds "brown" and "blue" the fishes with the gene T in duplex condition always have similar body color until about ten weeks after hatching, beginning in July, when the various brilliant colors of goldfish begin to appear.

Homozygous Transparent.—When the gene T' is in the duplex condition the fish has an appearance (plate 1, figure 3) entirely different from that of normal scaled fishes. The gene reduces the reflecting tissue so that it is either entirely, or nearly entirely absent on the body of the fish thus showing the red blood in the gills, the black pigment in the eyeballs, the white testis, the yellow eggs, and the black intestine visible to the eye without dissection. The melanophores are also greatly reduced to a black dotted area near the junction between the caudal peduncle and the caudal fin while on the other parts of the body the melanophores are either absent or, when present, form isolated dots very few in number. The presence of T' in duplex condition causes usually the complete loss of xanthophores. Occasionally xanthophores are present and form a yellow spot on one side of the trunk.

The expression of the genes varies in different individuals. Among the twenty-two homozygous transparent fishes the effects on the reflecting tissue varied as follows:

Five fishes were completely transparent, that is, without reflecting tissue altogether.

Fourteen fishes had a very small area of reflecting tissue situated on the ventral or the ventro-lateral parts of the abdomen.

Seven fishes had some reflecting tissue on one or both of the irises.

Four fishes had some reflecting tissue on a part of an operculum or a whole operculum but never on both opercula.

Three fishes had one to three normal scales. Nineteen fishes had no normal scale.

The effects on the melanophores and the xanthophores also varies in different individuals. Generally the melanophores are restricted to the junction between the caudal peduncle and caudal fin while the xanthophores are entirely absent. But I had also frequently observed that the melanophores are present on the basal area of the dorsal fin, on the basal area of the anal fin, or on the top of the head, and the xanthophores are present on a small area of the surface of the body usually beneath the dorsal fin.

Heterozygous Mottled.—The expression of the effects of a gene T and a gene T' in combination is more complex than when the genotypic constitution is TT or $T'T'$. The head is sometimes completely covered with the reflecting tissue, sometimes transparent in a small portion of the skin, or sometimes lacking the reflecting tissue in an entire operculum or even in an area as large as about one half of the surface of the head.

In the heterozygous mottled fishes there are always some normal scales mixed with the transparent scales. The number of normal scales in the 16 heterozygous fishes which I studied in detail varied from 2 to 18, being most frequently from 2 to 10.

In the body wall of the trunk the inner layer of the reflecting tissue is either entirely present or reduced to about two-thirds of the whole surface. Hence the heterozygous fish is never completely transparent.

The melanophores, xanthophores, and the bluish prismatic color are always mixed in the characteristic mottle condition and there is a greater number of the colored spots on the dorsal and the dorsolateral parts of the body than on the ventral parts.

WHY TRANSPARENT FISHES ARE RARE

In reviewing the books on goldfish published in Japan, such as that of MATRUBARA (1908), or in the United States such as that of INNES (1917), I found no records of the existence of the transparent fish as distinguished from the mottled fish, "shubunkin," or "calico." In the Chinese books on

goldfish I had also found no records of the transparent fish as a distinct breed. In my previous paper (CHEN 1925) I described such a fish but at that time I was not aware that such a fish might be different from the mottled fish in genotype and form a distinct breed. Altogether I mated nine transparent scaled fishes obtained from the local goldfish breeders at Nanking and found that all of them were heterozygous (families 1, 3, 4, 15, 16, 17). All these facts show that the transparent fish is rarely found in the goldfish aquarium of the ordinary goldfish breeders.

But in my breeding work I found that the proportion of the transparent fish in the F_4 generation is not less than one fourth. The transparent fish did not seem to have a poor viability as ordinary recessive breeds of animals or plants. I suggest the following three explanations to be the cause of the rarity of the transparent fish in the aquarium of the common goldfish breeders:

One explanation is that the heterozygous class being twice as numerous as the homozygous class has a greater chance to be obtained from the goldfish breeders. Another explanation is that the goldfish breeders often put five or six fishes into a breeding basin and frequently a normal scaled fish is mated with the transparent scaled fish with the result that most of the transparent scaled offspring are heterozygous. A third explanation is that during the classification and counting of the fishes of the F_4 generation containing the three different kinds of fishes I noticed that when the number of fishes in a basin amounted to several hundred with a keen competition of the fishes in the struggle for food, the transparent homozygous fish were always smaller than the other two classes of fish. The common practice of the goldfish breeder is to make a selection of the desirable fishes to rear when the young fishes reach the size of about 20 to 30 mm. At this time the larger fishes are selected to be grown for sale or for breeding and the smaller fishes are discarded or disposed of in other ways. If the transparent fishes are allowed to grow along with other fishes, it is probable that the other classes of fish will be selected in greater number and the transparent homozygous fish will be mostly discarded and only a small portion selected and grown for sale or for breeding. Probably, it is due to all the above mentioned reasons that the homozygous transparent fishes are rare and difficult to obtain from the common goldfish dealer and the existence of this class of fish has not yet been recorded in the goldfish books of any language.

SOMATIC MUTATIONS

In the summer of 1926 I found that among the offspring of the matings

between the normal scaled females and males there were six fishes which had a portion of their scales becoming transparent. But these fishes were different from the transparent scaled fishes which I studied. In the ordinary transparent scaled fishes the homozygous forms are nearly completely transparent and without chromatophores and the heterozygous fishes have the characteristic mottled pattern while in the case of the exceptional transparent scaled fishes produced from the mating of two normal scaled fishes the reflecting tissue is only partially reduced and yet the chromatophores do not form the characteristic mottled pattern. These exceptional transparent scaled fishes were of the ordinary red or white color like ordinary normal scaled fishes.

In the spring of 1927 I made two matings of the exceptional transparent scaled fishes with normal scaled fishes. These two matings (E3, E5) produced only normal scaled offspring. In the same breeding season I made one mating (1-9) of the exceptional transparent females and the exceptional males. The offspring obtained from this mating were also all normal scaled as the other normal scaled breeds of goldfish. This result proved that these exceptional transparent scaled fishes were genotypically with the TT genes but phenotypically with some transparent scales. Probably the formation of the transparent scales on the body of such fishes might be due to a somatic mutation of one of the gene T into a gene T' but the germplasm was not affected.

Among the forty fishes which I used to distinguish the homozygous from the heterozygous fish by means of the progeny tests I found one exceptional female fish ($B11$) which had one side of its body like an ordinary transparent homozygous fish but another side of its body like an ordinary heterozygous mottled fish with a non-transparent operculum and three normal scales, a thing which has never been found in the ordinary homozygous transparent fish. The mating of this fish with a normal scaled male fish produced offspring all belonging to the heterozygous mottled class thus proving that the fish was homozygous for the two $T'T'$ genes. The fact that one side of the body showed the heterozygous condition might be explained by a somatic mutation of one of the T' genes into a T gene during the early stage of development of the fish so that one side of the body was affected.

If the explanations of the above exceptional cases be true we might say that the gene T may mutate to gene T' in the somatic cells and the reverse mutation is also possible.

REVIEW OF LITERATURE AND COMPARISON WITH OTHER CASES

So far as I know there is only one paper on the inheritance of the characters of goldfish. This is the paper of HANCE (1922). In this paper HANCE touched upon the inheritance of colors, telescopic eyes, and the paired caudal fins and concluded, without reporting any data, that these characters were Mendelian. Although there was a picture of the mottled fish in his paper he seemed not to have worked or noticed the simple inheritance of this character. In my breeding work I found the inheritance of the color, telescopic eyes, and paired caudal fin, although with segregations in the F_2 generation, was very complicated whereas the inheritance of the mottled pattern was very simple and followed Mendel's law with the observed ratio very close to expectation. Probably this is the first case of Mendelian inheritance observed in the ordinary goldfish.

The inheritance of the transparency and mottling is a peculiar case of Mendelian inheritance in several respects. The heterozygous condition is a new type distinct from the two homozygous types. The new heterozygous character is a mosaic of the two homozygous types in regard to the presence and absence of reflecting tissue and chromatophores. The genes produce manifold effects on the reflecting tissue, melanophores, and xanthophores. A variegated condition is produced by the contrasting allelomorphic genes. Expression of the genes is somewhat variable. The combination of the above cited peculiarities in a single case is very rare. I found no parallel case in the genetics of animals or plants.

The case of the transparency and mottling is similar to that of the blue Andalusian fowl as reported by BATESON and PUNNET (BATESON 1911). In each case there is a distinct heterozygous type different from the two homozygous types the ordinary distinction of dominance and recessiveness being lacking. The heterozygous type in both cases is a sort of mosaic of the two homozygous types. In the goldfish the heterozygous type is mosaic in regard to the presence or absence of reflecting tissue and chromatophores. In the fowl, the heterozygous type is mosaic in regard to presence and absence of black pigment. However these two cases are not entirely similar since the genes of the goldfish have manifold effects while those of the fowl have only a single visible effect.

The case of transparency and mottling is more similar to that of the roan in the shorthorn cow (BATESON 1911). Here the red color in crossing with the white cow produces offspring with both red and white spots or roan just as the crossing of fishes with reflecting tissue distributed uniformly on the body with fishes lacking the reflecting tissue produces off-

spring with variegation in the presence or absence of the reflecting tissue. These two cases are also similar in the mosaic expression of the two homozygous types. However they are dissimilar in the presence of the manifold effects in the case of goldfish and the absence of it in the case of cattle.

SUMMARY

1. A pair of Mendelian factors has been definitely found in the goldfish. I propose to call the new character "transparent." This pair of factors is not sex-linked.

2. The homozygous condition of the transparent factor has the following manifold effects: the reflecting tissue is nearly lacking so that the red gills are visible through the operculum, the black retinal pigment is visible through the iris, and the white testis or yellow eggs are visible through the body walls. In such a fish the melanophores are restricted to a few black dots aggregated near the junction between the caudal peduncle and the caudal fin. The xanthophores are generally completely absent. The normal allelomorph of transparent when homozygous causes the production of the reflecting tissue and the two kinds of chromatophores to cover the whole body.

3. The heterozygous condition causes the production of the type of goldfish called "calico" in the United States and "shubunkin" in Japan. This heterozygous form is a mosaic of the two forms of homozygous fish in regard to the reflecting tissue, but the combined action of the two kinds of genes affects the chromatophores in a peculiar way so that a characteristic mottled pattern is produced.

4. In a strict sense there is no dominance and recessiveness between the members of this allelomorphic pair. But, according to the idea of MORGAN, the transparent condition might be said to be dominant and the normal condition to be recessive because the heterozygous mottled condition is sharply distinct from the normal form but is somewhat overlapping with the homozygous transparent condition especially when the fish are smaller than 20 mm in size.

5. Some cases of somatic mutation of the normal gene into the transparent gene have been observed and a case of somatic mutation of the transparent gene into the normal gene has also been observed.

6. Wild goldfishes have been successfully crossed with different kinds of fancy goldfish with the production of fertile offspring.

The above cited investigation was undertaken in the Biological Laboratory of the SCIENCE SOCIETY OF CHINA while I was occupying a position in the Department of Zoology in the College of Agriculture of the NATIONAL

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RELATIVE INFREQUENCY OF SOYBEAN VARIETIES HAVING ONLY ONE FACTOR FOR YELLOW COTYLEDON[1]

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In the soybean there are two cotyledon colors, yellow and green. Both types are green in immature soybeans, but as the beans ripen, the green pigment fades out in yellow-cotyledon varieties, and persists in green-cotyledon varieties. In inheritance, yellow differs from green in possessing a factor or factors that cause the green pigment to fade out as the beans mature.

In crosses between yellow and green cotyledon types, yellow proves to be dominant, and in F_2, a ratio of 3 yellow to 1 green, or 15 yellow to 1 green, is obtained according as one or two (duplicate) factors, D_1 and D_2, are involved.

In a former paper (WOODWORTH 1921) it was stated that while all the data available indicated that yellow color of the cotyledons was dominant to green, yellow cotyledon varieties differed among themselves with regard to the factors D_1 and D_2 that cause the green pigment to disappear at maturity. "Some, perhaps most varieties, as, for example, Auburn, possess only one factor of this nature, while others typified by Variety 8, possess two such factors." It now appears that the reverse of this statement is true, that is, that most yellow-cotyledon varieties possess two factors instead of only one. This is borne out by a study of the results secured to date from crosses showing segregation in cotyledon color (table 1). Of the 27 varieties listed in table 1, 23, or eighty-five percent, possess two factors for yellow cotyledon.

In contrast with this condition in soybeans, SHULL (1918) finds that types of shepherd's purse having duplication of a leaf-lobe factor are less frequent than those having only one such factor. From this fact he argues that "the duplication of this factor has taken place at a relatively recent date." Taking the same line of reasoning we may say that duplication of cotyledon factors in the soybean probably occurred at a remote date. The wild soybean, which is supposed to be the ancestor of our common

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types, has yellow cotyledons. The constitution of the wild soybean with respect to D_1 and D_2 has not been determined experimentally. However, the large number of common varieties descended from it and known to have both factors, is an indication that it also contains both. If soybeans with green cotyledons have arisen from the yellow by mutation, we should expect that single factor changes would occur more frequently

TABLE 1

Summarizing results of experiments on soybean crosses involving segregation in cotyledon color.

YELLOW COTYLEDON VARIETY	F_2 RATIO YELLOW TO GREEN COTYLEDON	YELLOW COTYLEDON VARIETY	F_2 RATIO YELLOW TO GREEN COTYLEDON
Mandarin[3]	14.3:1	Progeny 2214	11.7:1
Aksarben[3]	15.0:1	Progeny 2218	15.2:1
Ito San[3]	17.0:1	Progeny 2225	18.6:1
Jap. Variety[3] (J_1)	16.2:1	Progeny 2235	16.6:1
Manchu[3]	9.3:1	Progeny 2247	17.8:1
Green (C_1)[3]	18.8:1	Progeny 2261	17.3:1
Variety 8	7.9:1	Progeny 2265	14.9:1
Midwest	16.5:1	Progeny 2266	16.8:1
S. P. I. 65345	17.6:1	Progeny 2269	14.1:1
Ilsoy	10.5:1		
Wea	15.6:1	Auburn	3.2:1
Progeny 2199	11.3:1	Mikado	4.1:1
Progeny 2208	24.3:1	Wilson	3.8:1
Progeny 2213	18.8:1	Progeny 2262	3.1:1

[3] Data from OWEN (1927).

than changes involving more than one locus at a time. The occurrence of single-factor changes would result in the production of one-factor varieties as an intermediate stage from two-factor types to green. The scarcity of one-factor types, however, points to the conclusion that green-cotyledon varieties, if produced by mutation from the yellow, are the result, in most cases, of simultaneous changes in duplicate factors.

The occurrence of duplicate factors for a character can be accounted for by a doubling of chromosome number (tetraploidy) or by a doubling of only the particular pair of chromosomes carrying the original factor. In either case we should expect that other factors carried on the chromosomes involved would show duplication. In the problem under discussion, the factor G for green seed coat color, is linked with one of the factors for cotyledon color, either d_1 or d_2, with about 12.5 percent crossing over. If a doubling in chromosome number were responsible for duplication of cotyledon factors, we should expect to find duplicate factors for green seed coat color, one linked with d_1, the other with d_2. So far as known, there is no duplication of factor G.

Another possibility suggested by SHULL (1918) is the mating of non-homologous chromosomes. This would result in duplication, but also in the production of a single factor type through segregation and recombination. It would seem, therefore, that on this hypothesis the number of one-factor types would at least equal the number of two-factor types.

It appears that in nature there is very little or no natural crossing between yellow and green cotyledon varieties; for such crosses would lead to the production of one-factor yellow varieties as well as two-factor yellow varieties. It is a simple matter to produce one-factor varieties by isolating the segregates from hybrids heterozygous for two factors. Such hybrids produce both yellow and green cotyledon beans in a 15 to 1 ratio, and when the yellows are planted, we expect that 7 will breed true for yellow, 4 will segregate in a 3 to 1 ratio, and 4 in a 15 to 1 ratio. To establish a one-factor variety, we have only to test the yellows of a 3 to 1 ratio plant. Those that breed true for yellow are homozygous for one factor only. In every cross involving two factors, there should be two such types, one carrying D_1, the other carrying D_2. It is clear that if many crosses occurred between two-factor yellows and greens, many one-factor varieties would be produced. The infrequency of one-factor yellows in nature is therefore an argument against the occurrence of such natural crosses.

Simultaneous mutations involving duplicate genes at the same time are believed to be too rare to account for the number of green cotyledon varieties found in nature. Furthermore, some of these varieties show maternal inheritance of cotyledon color when crossed with yellows (TERAO 1918; PIPER and MORSE 1923; and OWEN 1927) and must, therefore, have been produced in some other way than by mutation. Also, the evidence is against the occurrence of many natural crosses between yellow and green varieties. There appears to be at present, therefore, no satisfactory explanation for the small number of one-factor yellow varieties as compared with the number of two-factor yellow varieties of soybeans.

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CHROMOSOME NUMBERS IN FUNCTIONING GERM CELLS OF SPECIES-HYBRIDS IN WHEAT[1]

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INTRODUCTION

The second and later generations of hybrids between tetraploid and hexaploid species of wheat have chromosome conditions which are very different from those which are to be expected on the basis of the numbers in F_1 gametes. The parental numbers appear far too frequently in F_2, and the intermediate numbers disappear in later generations. The reasons for these differences from the expected cannot be satisfactorily determined by the study of ordinary hybrids alone. But additional information, particularly in regard to the chromosome numbers of those gametes which are capable of functioning, can be obtained from the study of backcrosses.

KIHARA (1919, 1924) and SAX (1922a) have shown that in the F_1 the 14 chromosomes from the tetraploid parent (emmer series) mate with 14 from the hexaploid parent (*vulgare* series), leaving 7 from the latter unmated. These 7 univalents fail to divide at the homotypic division and move at random to either pole. The chromosome numbers of the gametes therefore vary between 14 and 21, and the frequencies of the various numbers should be 1, 7, 21, 35, 35, 21, 7, 1. Consequently the great majority of plants in F_2 and in later generations should have intermediate numbers.

But several authors have shown that segregates with 28 and 42 chromosomes (somatic) are numerous, that many others have 29, 30, 40 or 41,

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and that the proportion of plants with approximately 35 is very small in comparison with expectations (KIHARA 1921, 1924; SAX 1923; THOMPSON 1925; THOMPSON and HOLLINGSHEAD 1927). Furthermore those intermediate numbers which were present in F_2 were shown to disappear in later generations. In addition KIHARA (1924) found that the chromosome formula of plants with more than 14 bivalents must be written $(14+x)$ bivalents $+(7-x)$ univalents. If any plant has more than 14 bivalents it has also enough univalents to make the total number 21. Such combinations as 15 bivalents plus 0 to 5 univalents, 16 plus 0 to 4, etc., do not appear, or only very rarely, although they should be expected fairly frequently, particularly in certain F_3 progenies.

Different suggestions were made by the authors mentioned to account for these results. SAX thought that they were due to the failure of the intermediate classes of gametes to function owing to chromosome unbalance. KIHARA attributed them rather to the failure of certain classes of zygotes to develop. There are other possible factors such as the loss of univalent chromosomes through lagging, chromosome conditions in the endosperm, some peculiar method of chromosome distribution, or some favorable relationship between chromosome numbers in male and female cells.

In order to decide the exact cause, it is necessary to know the chromosome constitution of the gametes which are capable of functioning. By means of counts in F_2, it is possible to determine the total number in the 2 gametes which unite to produce an F_2 plant but not the number in individual gametes. But by using backcross material this information can be obtained. When the chromosome number of the backcross has been determined, and that of the pure parent is known, that of the F_1 gamete which functioned can easily be inferred.

KIHARA (1925) has reported some results of backcrossing *T. Spelta* × *T. polonicum* F_1 male with the pure parents. Of the backcross with *polonicum* 4 plants were examined and all were shown to have resulted from the functioning of F_1 male gametes with 14 chromosomes. Of the backcross with *Spelta*, 10 plants were examined and 8 were shown to have resulted from the functioning of F_1 male gametes with 19 to 21 chromosomes. Since *polonicum* has 14 and *Spelta* 21, KIHARA concluded that the pollen grains which function are those which have a chromosome number similar to that of the female. It is possible, for example, that a 14-chromosome pollen tube may not develop as well as a 21-chromosome tube in a stigma of a plant with 21 chromosomes.

WATKINS (1927) has reported counts on backcrosses with *turgidum* ×

vulgare which lead him to conclude that all F_1 female gametes are capable of functioning, but that there is much elimination of male gametes.

METHODS

All chromosome counts were made on pollen mother-cells of stamens fixed in a mixture of absolute alcohol and acetic acid (2:1) and examined in aceto-carmine. Such preparations are much better than paraffin sections for the study of univalents, though usually not for total counts if the numbers are large. In this case the determination of the number of univalents is all that is necessary since if the backcross is with the 14-chromosome parent the number of univalents is the same as in the F_1 gamete which functioned (all 14 from the pure parent mating with 14 from the F_1 gamete); and if the backcross is with the 21-chromosome parent, the number of univalents is 7 minus the number in the F_1 gamete (each univalent from the latter finding a mate among the extra 7 chromosomes from the *vulgare* parent). Nevertheless, whenever it was possible—and this was the case in nearly every plant—the total number of bivalents and univalents was also determined for confirmation.

The number of univalents can be determined most easily and accurately at the heterotypic anaphase since they lag behind the bivalents, and since in aceto-carmine preparations of fixed material, side views are usually obtained. The number visible in the different pollen mother-cells of the same plant varies somewhat owing to premature splitting, failure to move into the equatorial position, or entanglement with the bivalents. But when any considerable number of cells is available for observation the true number appears in a large proportion of cases, as can easily be confirmed from total counts. At heterotypic metaphase, although most of the univalents are off the plate and can be recognized, some of them may be at the edge so that accurate determination of them is more difficult than at anaphase, though usually not impossible. In a few cases a small proportion of the cells showed 1 or 2 univalents more than was definitely determined to be the true number. This was apparently due to the occasional failure of 1 of the primary 14 pairs to mate. Many of the counts were made on material raised in the greenhouse during the winter of 1926–27, and the rest on material raised in the field in 1927.

Four kinds of F_1 plants were used: *dicoccum-vulgare* 1, *durum-vulgare* 1, *durum-vulgare* 2, and *dicoccoides-vulgare* 1. *Vulgare* 1 is the variety Marquis; *vulgare* 2 is of Chinese origin; the *dicoccum* variety is a white spring emmer; the *durum* is Iumillo; the *dicoccoides* is wild emmer. The *vulgares* have 21 chromosomes and each of the others 14.

EXPERIMENTAL DATA

Table 1 gives the cytological findings for a number of kinds of back crosses. It shows the frequencies of the F_1 gametes, with the various numbers of univalents, which functioned to produce the backcrosses. The total number of chromosomes in these gametes is obtained by adding 14 (the bivalents) to the numbers 0 to 7 in the first line of the table. Both male and female gametes of each of the 4 kinds of F_1 were investigated by using F_1 as both pollen and seed parent in the back-crossing. Also in the case of the *durum-vulgare* F_1, backcrosses were made with both *durum* and *vulgare* parents. The data on the F_1 gametes which functioned in all these kinds of backcrosses are given in the table.

In the backcrosses with *vulgare*, the number of univalents actually seen in each case was 7 minus the number shown, since each univalent in the F_1 gamete would mate with one of the 7 extra chromosomes from the *vulgare* parent and the rest of the 7 would be left unmated.

A study of table 1 brings out certain conclusions which may now be discussed in order.

TABLE 1

Frequencies of functioning F_1 gametes with various numbers of univalent chromosomes.

BACKCROSS	UNIVALENTS IN F_1 GAMETES								TOTALS
	0	1	2	3	4	5	6	7	
1. *Dicoccum* ♀ × (*vulgare* 1-*dicoccum*) F_1 ♂	18	8	2	3	2	0	2	2	37
2. *Dicoccum* ♂ × (*vulgare* 1-*dicoccum*) F_1 ♀	10	6	5	6	2	0	0	0	29
3. *Durum* ♀ × (*vulgare* 1-*durum*) F_1 ♂	12	7	4	2	0	0	1	3	29
4. *Durum* ♂ × (*vulgare* 1-*durum*) F_1 ♀	14	3	4	2	4	2	1	2	32
5. *Durum* ♀ × (*vulgare* 2-*durum*) F_1 ♂	3	2	0	1	2	2	0	5	15
6. *Durum* ♂ × (*vulgare* 2-*durum*) F_1 ♀	1	1	3	6	4	2	2	1	20
7. *Vulgare* 1 ♀ × (*vulgare* 1-*durum*) F_1 ♂	8	4	2	1	1	1	2	5	24
8. *Vulgare* 1 ♂ × (*vulgare* 1-*durum*) F_1 ♀	5	2	3	2	2	3	0	0	17
9. *Vulgare* 1 ♀ × (*vulgare* 1-*dicoccoides*) F_1 ♂	2	1	2	1	0	1	1	1	9
10. *Vulgare* 1 ♂ × (*vulgare* 1-*dicoccoides*) F_1 ♀	2	1	1	2	1	1	0	0	8
11. *Vulgare* 2 ♂ × (*vulgare* 2-*durum*) F_1 ♀	0	0	2	2	3	1	0	1	9
Expected proportions	*1*	*7*	*21*	*35*	*35*	*21*	*7*	*1*	

THE FAILURE TO FUNCTION OF MANY GAMETES WITH INTERMEDIATE CHROMOSOME NUMBERS

The last line of the table shows the proportions of the various classes of gametes which are to be expected if all are capable of functioning. The actual proportions are very different in every case. The most conspicuous difference is the small number of gametes with intermediate numbers (3, 4, and 5). For example, the ratio of gametes with 0 to those with 3 is expected to be 1:35, whereas it is actually 18:3, 10:6, 12:2, etc. A result of this kind would be anticipated from the work of several investigators on F_2. SAX (1923) believes that nearly all F_1 gametes with intermediate numbers are sterile. KIHARA (1924), on the other hand, thinks they are all capable of functioning but that their failure to produce offspring is due to zygotic mortality. These results strongly favor the theory of gametic sterility. Nevertheless a high enough proportion of them is capable of functioning to produce many F_2 plants. Presumably their usual failure to function is due to the bad effects of an unbalanced chromosome number. Whether they actually die or are merely unsuccessful in competition with the others is not determined. The percentage of visibly abortive pollen in F_1 is not high enough to account for these figures.

In a later publication (1925) KIHARA reported data which indicated that the functioning of the pollen grain may depend on the relation of its chromosome number to that of the female plant. When *Spelta*×*polonicum* F_1 male was backcrossed with *polonicum* female, all the 4 F_1 pollen grains which functioned to produce the plants examined, had 14 chromosomes; but when the backcross was with *Spelta* 8 of the 10 grains which functioned had from 19 to 21 chromosomes. KIHARA concluded that the pollen grains which function are those which have a chromosome number similar to that of the female. Failure to function may result from poor growth of the pollen tube in a stigma which is unsuitable because of its chromosomes.

Our results do not support this conclusion. In the backcross with *durum* female (line 3 of table 1) 19 out of 29 grains had 14 or 15 chromosomes and 4 had 20 or 21; in the backcross with *vulgare* female (line 7) 12 out of 24 grains had 14 or 15 and 7 had 20 or 21. Grains with the higher numbers were, therefore, only slightly more successful on *vulgare* than on *durum* stigmas. Half the grains which were successful in the *vulgare* backcross had only 14 or 15 chromosomes.

PREPONDERANCE OF 14- OVER 21-CHROMOSOME GAMETES

Chromosome unbalance will not account for the great preponderance, in nearly every case, of gametes with no univalents, or only 1, over those

with 6 or 7. It will be observed from the table that the ratio of gametes with 0 univalents to those with 7 in the different cases is 18:2, 10:0, 12:3, etc., whereas it should be 1:1. This is doubtless due, in part at least, to the loss of chromosomes through lagging at the reduction divisions. These laggards frequently fail to become incorporated in the new nucleus and degenerate in the cytoplasm. (For the frequency of this phenomenon see THOMPSON and HOLLINGSHEAD 1927). Intermediate numbers would thus tend to become low, and high numbers to become intermediate (a condition which rarely functions). Whether this is the correct explanation or not, there are certainly far more functional gametes with 14 than with 21 chromosomes. This results in the great preponderance of emmer types in F_2 noted by THOMPSON and HOLLINGSHEAD in *dicoccum-vulgare* and to a less extent by THOMPSON (1925) in *durum-vulgare* hybrids.

One exception to this rule should be noted. When *vulgare* 2 is used in making *durum-vulgare* crosses, the F_1 gametes with 7 univalents are as numerous as those with 0 or more numerous.

DIFFERENCES BETWEEN MALE AND FEMALE GAMETES

Another important point evident in the table is that male gametes with intermediate numbers are eliminated in greater proportions than are female gametes with the same numbers. In nearly every case there is a smaller proportion with intermediate numbers when the F_1 is the male parent than when it is the female parent. This may be due to the greater competition among the numerous male gametes, only the stronger ones usually functioning. In no case, however, are all the males with intermediate numbers eliminated. And in no case are all the female gametes functional. In every case in which the F_1 was the female parent, the proportions of gametes with intermediate numbers are less than the theoretical. The only case in which the expected numbers are approached is where *vulgare* 2 was one parent.

This result is quite different from that obtained by WATKINS (1927) with *vulgare*×*turgidum* hybrids. He is of the opinion that all female F_1 gametes in that cross are capable of functioning. The correctness of our conclusion, for our material at least, is confirmed by comparing the percentage of seed set in backcrossing where the F_1 was the female parent, with that set in straight crossing. The figures in this connection are given in table 2. In every case the percentage of seed set is considerably less in the backcross, showing that a higher proportion of the eggs of F_1 than of the pure parent fails to function. These crosses were all made in the same year by the same person.

TABLE 2

Percentages of pollinated flowers setting seed.

Vulgare-durum F_1 ♀ × *durum* ♂	34.46
Vulgare-durum F_1 ♀ × *vulgare* ♂	33.96
Vulgare-dicoccum F_1 ♀ × *dicoccum* ♂	33.40
Vulgare-dicoccum F_1 ♀ × *vulgare* ♂	34.44
Vulgare ♀ × *durum* ♂	72.87
Vulgare ♀ × *dicoccoides* ♂	59.21
Dicoccum ♀ × *vulgare* ♂	84.33

DIFFERENCES AMONG THE KINDS OF CROSSES

It is also evident from table 1 that somewhat different results are obtained with different kinds of F_1. For example there is a greater elimination of intermediate numbers in *vulgare-dicoccum* than in other F_1's. When *vulgare* 2 is used in crossing with *durum* the results are very different from those obtained when *vulgare* 1 is used. In the former case there is much less elimination of intermediate numbers. Its female gametes (backcrosses number 6 and 11) approach the expected much more closely than any of the others and both male and female gametes have 6 or 7 univalents much more frequently than do those of other crosses. Not only the kind of F_1 but also the kind of backcross is important. The elimination of both intermediate and high numbers of univalents is less when the backcross is made to *vulgare* than when it is made to *durum*. All these differences emphasize the danger in drawing sweeping conclusions from work with a limited variety of material. In the difficult problems of practical breeding with wheat, what is found to be impossible to accomplish with one or several crosses, may prove quite feasible with others.

CHROMOSOME NUMBERS IN RELATION TO ENDOSPERM DEVELOPMENT AND SEED MORTALITY

Certain aspects of these results have been determined or influenced by differences in the germinating capacity of different classes of seeds. This is illustrated in table 3 which shows the percentages of germination of grains from certain reciprocal backcrosses as well as from straight crosses. The grains recorded are those produced directly on the plants which were artificially pollinated, not on their offspring. It is clear from these data that in backcrosses with the 14-chromosome parent the germination is better, in some cases much better, when the F_1 is the female, than when it is the male parent. Even in straight crosses there is a

marked difference in germination depending on the direction of the cross. If the 21-chromosome parent is the female the germination is much higher than if it is male.

TABLE 3

Comparative germination in reciprocal crosses and backcrosses.

	PLANTED	GERMINATED	PERCENT GERMINATED
Dicoccum ♀ × F_1 ♂	72	48	66.6
Dicoccum ♂ × F_1 ♀	67	52	77.6
Durum ♀ × F_1 ♂	40	21	47.5
Durum ♀ × F_1 ♀	31	28	90.4
Vulgare ♀ × F_1 (*vulgare-durum*) ♂	29	26	89.6
Vulgare ♂ × F_1 (*vulgare-durum*) ♀	38	23	60.5
Vulgare ♀ × F_1 (*vulgare-dicoccoides*) ♂	17	17	100.0
Vulgare ♂ × F_1 (*vulgare-dicoccoides*) ♀	22	12	54.5
Durum ♀ × *vulgare* ♂	93	51	55.9
Durum ♂ × *vulgare* ♀	19	16	84.2
Dicoccum ♂ × *vulgare* ♀	10	8	80.0
Durum ♀ × *Spelta* ♂	24	8	33.3

Note: These data are for directly crossed seeds, not for seeds borne on plants resulting from crossing. The seeds recorded in the first 4 sections of the table would produce backcross plants; those in the last 2 sections would produce F_1.

These differences in germination depend directly on the different degrees of endosperm development which are evident from the varying amounts of shrivelling of the seeds. And the endosperm development depends on chromosome conditions, as has been pointed out by WATKINS (1927). The chromosome number in the endosperm of seeds resulting from crossing a 21- with a 14-chromosome wheat will depend on the direction in which the cross is made. If the 21-chromosome plant is the female, the endosperm will have $56(21\times2+14)$ chromosomes since it is formed from the fusion of 2 female nuclei (each with 21) and a male nucleus (with 14). But if the 21-chromosome plant is the male, the endosperm will have $49(14\times2+21)$. In the one case the extra 7 *vulgare* chromosomes are diploid and in the other haploid. From the data on straight crosses given in table 3, it is clear that germination is much better when the 7 are diploid. Similar considerations must be kept in mind in discussing F_1 and plants which may have between 14 and 21 chromosomes.

TABLE 4

Chromosome numbers and endosperm development.

		LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED	TOTALS
Vulgare 1 ♀ × *vulgare* 1-*durum* F_1 ♂	Frequency	32	48		10	7	97
	Planted	10	17		2		29
	Died	2	1		0		3
	Univalents in F_1 gametes	7, 7, 7, 7, 7, 6, 6, 5	4, 3, 2, 2, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0		1, 0		
Vulgare 1 ♂ × *vulgare* 1-*durum* F_1 ♀	Frequency	11	2	44	12	20	89
	Planted	8	2	23	5		38
	Died	0	1	10	4		15
	Univalents in F_1 gametes	2, 3, 4, 4, 5, 5		0, 0, 0, 0, 0, 1, 2, 2, 2, 3, 4			
Vulgare 2 ♂ × *vulgare* 2-*durum* F_1 ♀	Frequency	8	5	26	16	21	76
	Planted	7	5	10	3		23
	Died	0	0	6	3		9
	Univalents	3, 4, 5, 5, 7	4, 4	2, 2			
Vulgare ♀ × *vulgare-dicoccoides* ♂	Frequency	10	14				24
	Planted	8	9				17
	Died	0	0				0
	Univalents	3, 4, 5, 6, 7	0, 0, 1, 2				
Vulgare ♂ × *vulgare-dicoccoides* ♀	Frequency	1		15		15	31
	Planted	1		14		7	22
	Died	0		4		4	8
	Univalents			0, 0, 3, 3, 4, 5		1, 2	
Durum ♂ × *vulgare-durum* ♀	Frequency	24	15				39
	Planted	8	3				11
	Died	0	0				0
	Univalents	0, 0, 1, 2, 6	4, 4				

Information regarding the interrelationships between chromosome numbers, the direction of crossing, endosperm development, and germination is given in table 4. If one compares the first two sections of the table, it is seen that in backcrossing with the 21-chromosome parent, the proportion of wrinkled and shrivelled seeds obtained is low when the F_1 is the male parent but very high when F_1 is the female. In the former case (*vulgare* ♀ × F_1 ♂) the endosperm has the extra 7 diploid plus whatever number of univalents the male contributes, which is usually 0 *or* 7. And such endosperm is almost always well developed. Therefore the endosperm develops well if the 7 are exactly diploid or triploid or nearly so. From the chromosome numbers determined (bottom of first section of table 4) it is also evident that the triploid condition gives better endosperm than the diploid, since when the male contributes 6 or 7 univalents the seeds are large, whereas when it contributes 0, 1 or 2, they are small (though not shrivelled).

In the reciprocal of this cross (F_1 ♀ × *vulgare* ♂; second section of the table) the endosperm contains in the diploid condition whatever chromosomes are contributed by the F_1 (usually less than 7) plus the complete set of 7 contributed by the *vulgare* male. Consequently the endosperm is usually haploid for all 7 (when F_1 contributes 0 univalents) and frequently triploid with respect to some chromosomes and haploid with respect to the rest. Under these conditions endosperm development and germination is usually poor. Moreover the actual chromosome counts (bottom of second and third sections) show that the small proportion of round seeds do have the triploid condition of most of the chromosomes while the wrinkled ones have the haploid.

A similar situation is seen in the backcross of *vulgare-dicoccoides* with *vulgare*.

When the F_1 is backcrossed with the *durum* the seeds are plump. In this case the extra 7 *vulgare* chromosomes are rarely represented at all in the endosperm, since the F_1 usually contributes none, and of course the *durum* has none. Therefore the endosperm is like that of pure *durum*.

All these cases are in line with a general rule which may be stated as follows: The endosperm is well developed when it is (a) entirely without the extra 7 *vulgare* chromosomes, (b) when it has either 2, or, (c) better still 3 complete sets. It is likely to be badly developed when it is (a) haploid for all 7 or (b) diploid or triploid for some only of the 7 or (c) diploid of triploid for some and haploid for others. In general the endosperm appears to be affected in the same way as the gametes by unbalanced chromosome numbers; the unbalance may be even greater in the endosperm.

A number of important applications of these conclusions may be pointed out. In ordinary crossing one obtains many more F_1 plants for the same amount of labor if *vulgare* is the female than if it is the male parent, since in the latter case the endosperm is haploid with respect to the seven. This is important in practical breeding where a large F_2 may be required. Again it is well known that certain wide crosses, for example wheat×rye, wheat×Aegilops, *vulgare*×*monococcum*, etc., can be made in only one direction. The species with the larger chromosome number must be used as the female, in order to give an endosperm which is diploid for the extra chromosomes.

The conclusions reached above make it probable also that the F_2 and later generations usually raised are not fair samples. There is a tendency to select the better looking seeds for planting and to leave the shrivelled ones or to take no special care with them. But the embryos of the better seeds are likely to have either 0 or all 7 of the extra chromosomes while the shrievelled ones are likely to have the intermediate numbers. It is therefore probable that the lack of intermediate chromosome numbers is due in part to the conscious or unconscious selection of the better seeds for planting. Of course a proportion of the poor seeds would fail to germinate in any case, particularly if conditions were not favorable. Nevertheless the frequency of plants with intermediate chromosome numbers would be increased by giving special attentation to the poorer seeds. And it is probable that such plants are the ones most likely to possess the combinations of characters which the practical breeder desires.

SAX (1922b) has reported some results which are in agreement with this conclusion. In a cross between Kubanka and Bluestem he found that a majority of F_2 seeds (borne on F_1 plants) which failed to germinate were in the lower weight classes. He also found a correlation of 0.24 between wrinkling of F_2 seeds (borne on F_1) and sterility of F_2 plants which produced heads. He could find no correlation, however, between wrinkling and size of F_2 plants.

It is probable that the frequency of intermediate numbers in table 1 would have been increased by selection of wrinkled seeds and special care in their germination. These facts about the effect of chromosome numbers in endosperm development and germination were not realized when the seeds of some of the backcrosses were planted. In some cases, however, all the seeds available were planted, but no special care was taken with the shrivelled ones.

BEARING ON RESULTS PREVIOUSLY REPORTED FOR F_2 AND LATER GENERATIONS

The chromosome numbers in F_2 and later generations as reported by SAX (1923), KIHARA (1924), THOMPSON (1925), THOMPSON and HOLLINGSHEAD (1927), are in general such as would be expected if the F_1 gametes which are capable of functioning have the chromosome numbers reported in this paper. One discrepancy is a frequency of plants with intermediate numbers lower even than our figures would indicate. This may be explained in part by the selection of the better seeds for planting. It may also be due in part to the greater effect of chromosome unbalance in the endosperm in the ordinary hybrids than in the backcrosses. In the latter one parent contributes 1 or 2 complete sets of the 7 or none at all, whereas in the former the F_1 makes all 3 contributions of intermediate numbers to the endosperm. The effect of chromosome unbalance is therefore likely to be greater in the endosperm of ordinary hybrids than in that of backcrosses.

Another marked discrepancy is the absence from F_2 of any plants with more than 14 bivalents and less than 21 as the sum of bivalents and univalents. Such combinations as 15 bivalents plus 0–5 univalents, 16 plus 0–4, etc., do not appear or only very rarely. They should be found if the gametes have the numbers shown in table 1. They should not be very frequent in F_2 but they should occur, and they should be fairly frequent in certain F_3 families. This study shows that their absence is not due entirely to gametic elimination. It must be due also to zygotic mortality. Apparently zygotes having more than 14 bivalents without having enough univalents to make the total 21 are not viable. They may die as a result of endosperm abortion. They could result only if both gametes had an intermediate number of univalents. Therefore the endosperm could not be triploid or even diploid for all 7 but would be triploid for some of the 7. For example if the pollen grain had chromosomes a and b of the extra 7, and the egg had a, b and c, then the endosperm would have 3 a's, 3 b's and 2 c's, and none of the others. It has been shown from the data on backcrosses that such an endosperm would be severely shrunken and the seed probably fail to germinate or die early. Hence the non-appearance of such combinations may be due to endosperm abortion. Of course the young zygotes may die from other causes.

SUMMARY

1. An effort has been made, by the study of backcrosses, to determine the causes of the cytological conditions which have been reported for

hybrids between 14- and 21-chromosome wheats. The study of backcrosses makes possible the exact determination of the chromosome numbers of F_1 gametes which are capable of functioning. This information is not obtainable from the study of ordinary crosses. Four kinds of F_1 were backcrossed reciprocally so that both male and female gametes could be investigated and both 14- and 21-chromosome parents were employed in making the backcrosses.

2. In nearly every case the gametes with numbers of extra *vulgare* chromosomes intermediate between 0 and 7 were in much smaller proportions than were to be expected, if all gametes are capable of functioning.

3. In most cases the gametes with no extra univalent chromosomes (14 in all) were much more numerous than those with 7 (21 in all).

4. There are marked differences between the male and female gametes of F_1, the latter showing less elimination of intermediate numbers than the former, though by no means all eggs are capable of functioning and a proportion of functioning pollen grains do have intermediate numbers.

5. There are marked differences between the different kinds of hybrids with respect to their functional gametes, and slight differences depending on whether a hybrid was backcrossed with the *vulgare* or with the emmer-type parent. It is dangerous to draw sweeping conclusions from a limited variety of material.

6. The endosperm is likely to be poorly developed (and the seeds shrivelled) unless its extra 7 *vulgare* chromosomes are completely absent, or completely diploid, or completely triploid. The farther it departs from these conditions the greater is the shrivelling. Reciprocal crosses may, therefore, give very different results and wide crosses may be possible in only one direction. Unless special care is taken of the shrivelled seeds in species-crosses a fair sample is not obtained in F_2 since the shrivelled ones contain chromosome combinations not likely to be found in plump ones.

7. If gametes with the frequencies found mate at random, the results should be in general like those which have been reported for F_2. Discrepancies are seen in the lower frequencies of intermediate numbers and especially in the absence of combinations of more than 14 bivalents with less than enough univalents to make 21 altogether. The absence of these may be due to zygotic weakness but may also be attributed to endosperm abortion.

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INHERITANCE OF EARLOBE COLOR IN POULTRY[1]

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INTRODUCTION

The earlobe of the chicken is a structure on the skin of the face just below the ear, the outline of which is marked by a slight thickening of the tissues. It is bare of feathers and may be the same color as the rest of the face for which the degree of redness is somewhat dependent upon the health of the bird. As is true of most of the head furnishings, this structure is larger in males than females.

For some breeds of poultry, more commonly those classified as Mediterraneans, the color of the earlobe differs from that of the rest of the skin of the face. In such cases it is of a pearl white color and although the histology of the tissue has not been studied the color appears to be due to a deposition of white material just beneath the epidermal layer. In the White-Faced Black Spanish breed the white color is extended to cover the entire face. In the breeds of the Mediterranean group the earlobe is proportionately larger than in most other breeds.

The poultry fancier has decreed that the earlobe must be all white or all red, making the color a breed characteristic. Since even slight variations from standard color constitute a show ring disqualification, uniformity of color has been rigidly selected for during a considerable period. For this reason most breeds are quite constant for earlobe color.

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Although the earlobe is one of the conspicuous furnishings of the head of the chicken, we find little mention of it in genetic literature. Numerous crosses have been made involving differences in earlobe color but practically nothing has been written upon the inheritance of its color variations. The variability of the F_1 generation and the complex nature of its factorial basis have probably been the conditions which have discouraged the undertaking of genetic investigations of this character.

This study is an attmpt to analyze as completely as our knowledge of the genetics of poultry will permit, the factorial basis of earlobe color.

BREEDS USED

Most of the conclusions of this paper are based upon the results of crosses of the Jersey Black Giant and the Single Comb White Leghorn breeds. However, in the course of other genetic studies, crosses of several breeds differing in earlobe color have been made and in all cases accurate records have been kept. The white-earlobe breeds used in this study were the Single Comb White Leghorn, Rose Comb Brown Leghorn, and Single Comb Buff Leghorn. The red-earlobe breeds were Jersey Black Giant, Barred Plymouth Rock, Single Comb Rhode Island Red, Dark Brahma, White Wyandotte and Silver Laced Wyandotte. The tables presented in the following discussion will indicate the crosses made of the various breeds.

CLASSIFICATION OF EARLOBE COLOR

Records of earlobe color were made at three months and six months of age. It was found that the description for the three-month age was not entirely reliable since changes in color sometimes took place afterwards. The description at the age of six months was used for classification since color attained at that age appeared to be the normal adult color. Any changes after this age were usually due to injury. The fact that only those individuals raised to the adult condition could be classified, accounts for the small numbers in some crosses.

The resulting offspring exhibited all gradations between entirely red and entirely white earlobes. For purposes of classification it became necessary to establish arbitrary groupings of the intermediate colors. Four grades were recognized; namely, white, predominately white, predominately red, and red. It is realized that there is some experimental error in using so crude a system of classification, but since it was the more accurately defined first and last grades that were of primary interest it seemed to satisfy the needs of the experiment at hand. The intermediate grades were not cases of blending of the red and white but the mingling

of the two in varying proportions. The variations are probably quantitative ones depending upon the amount of white tissue deposited and the relative amounts of white and red are determined by the proportion of the surface of the earlobe covered by the white.

F_1 GENERATION

For study of the results of mating red and white earlobe breeds of poultry, data were available from ten different crosses. In table 1 are

TABLE 1

Distribution of earlobe color for the F_1 generation of various crosses.

Mating	Females				Males			
	White	Predominately white	Predominately red	Red	White	Predominately white	Predominately red	Red
RED EARLOBE MALES BY WHITE EARLOBE FEMALES								
1 Black Giant male by White Leghorn female	1	20	27	2	2	17	24	1
2 Rhode Island Red male by White Leghorn female		2	21	1	1	1	19	3
3 Dark Brahma male by Brown Leghorn female	3	7	6	1	2	3	7	5
4 Barred Rock male by Brown Leghorn female	1	1	5	3	1	1		2
5 Barred Rock male by Buff Leghorn female	1	5	9	1	1	9	9	7
6 White Wyandotte male by Buff Leghorn female	6	2	1		1	7	1	
WHITE EARLOBE MALES BY RED EARLOBE FEMALES								
7 White Leghorn male by Black Giant female	12	11	6	1	12	11	7	
8 Brown Leghorn male by Silver Laced Wyandotte female.	3	4	5		3	3	5	2
9 Brown Leghorn male by Silver Pencilled Rock female	6	6	1		2	11	7	
10 White Leghorn male by Rhode Island Red female		9	19	3			14	9

shown the results of these crosses, six of which are matings of red earlobe males by white earlobe females and four in which the reciprocal relations were true. In two crosses, Jersey Black Giant by White Leghorn and Rhode Island Red by White Leghorn, reciprocal crosses of the same mating were available. A glance at table 1 will show that complete dominance of either color was not obtained. Also it is seen that the different matings of the same type (involving different breeds) are somewhat variable with respect to the proportions of red, white and intermediate colors. The small number of individuals involved in some matings may be responsible for the variability but later generations indicate that the various breeds may differ as to their genetic constitution for genes affecting earlobe color.

Variability of the F_1 Generation

In most crosses it is seen that all four grades of earlobe color are found in the F_1 generation. It is of interest to determine whether these variations are due to variability in dominace or lack of homozygosity. Either or both conditions may contribute to the variability of the F_1 individuals. Since a large number of F_1 individuals were not mated, only a limited amount of data are available for determining whether F_1 individuals differing in earlobe color actually differ in genetic constitution. From the cross of Jersey Black Giant by White Leghorn three F_1 females were mated to the same male. One of these females had red earlobes while the earlobes of the other two were predominately white. Only female offspring are listed here since it will later be shown that sex-linked factors are also involved in this cross. The ratios of the earlobe colors of the female offspring were as follows:

Hen Number	*White*	*Predominately white*	*Predominately red*	*Red*
5388–Red Earlobe		7	6	1
5415–Predominately White	3	3	1	
5452–Predominately White	3	9	2	

It will be seen that the offspring differ in accordance with differences among the F_1 parents.

Two F_1 males were backcrossed to several Jersey Black Giant females. One having predominately red earlobes produced female offspring which were classified as follows; 14 predominately white; 54 predominately red and 28 red. The other which had predominately white earlobes gave the following distribution of earlobe colors; 10 predominately white, 23 predominately red and 7 red. Here again the distribution of color among the offspring differed in accordance with the difference between the two F_1 males.

In the cross of Brown Leghorn by Silver Penciled Rocks two F_1 males were used. They were full brothers but one had earlobes classified as white and the other as predominately white. For certain other genetic studies, the two males were each mated to F_1 females from cross of Brown Leghorn by Buff Leghorn and Brown Leghorn by Rhode Island Red. In table 2 the results of these matings are shown. Male 987M which had

TABLE 2

Variability of earlobe color of F_1 individuals. Results of mating two F_1 males differing in earlobe color to similar lots of females; showing that the variability in the F_1 generation is due to differences in their genetic constitution.

Male Number	Females				Males			
	White	Predominately white	Predominately red	Red	White	Predominately white	Predominately red	Red
MATED TO F_1 FEMALES FROM BROWN LEGHORN—BUFF LEGHORN CROSS								
987M (white earlobe)	53				42			
989M (predominately white)	21	3	1		52	3		
MATED TO F_1 FEMALES FROM BROWN LEGHORN—RHODE ISLAND RED CROSS								
987M	38	9	2	1	31	3		
989M	21	16	4	2	20	9	3	

the entirely white earlobes produced offspring whose earlobes showed a greater amount of white than did the progeny of 989M. In the first mating shown in table 2 the autosomal constitution of the offspring was such that most of the earlobes were white but the few which were not entirely white came from male 989 M. In the second mating more earlobes appeared which showed varying degrees of red and here the offspring of 989 M had earlobes which averaged considerably more red. Since these two males were full brothers the difference in their genetic constitution with respect to factors influencing earlobe color must have been due to heterozygosity for these factors on the part of one or both parents.

The foregoing data seem to indicate that the offspring of F_1 individuals varying in earlobe color, tend to vary in the same direction as their parents. This may be due to the fact that the parental stock was not entirely homozygous for the factors involved and that we are dealing with a multiple factor situation. Since the breeds used seldom showed by their own variability any indication of heterozygosity it would be

difficult to establish homozygosity before crossing. These factors although exerting no apparent influence in the pure breed, did appear to be effective when the other factors were in a heterozygous condition.

SEX-LINKED FACTORS

Reciprocal F_1 matings of the same cross may be utilized to determine whether factors influencing earlobe color are carried by the sex-chromosome. In two crosses, White Leghorn by Jersey Black Giant and White Leghorn by Rhode Island Red, data from reciprocal crosses are available for comparison. Variability of the genetic constitution of individuals of a breed is here a source of error but it is believed that individuals of a single strain do not differ greatly in their constitution with respect to the major factors for earlobe color. If sex-linked factors affecting earlobe color are operative we would expect the female offspring of reciprocal crosses to differ. Since in the chicken, the male is the homogametic and the female the heterogametic sex, the female offspring of any cross receive their only Z- or sex-chromosome from the father. Since the female has only one Z-chromosome she should exhibit all sex-linked characters of her father. Thus if there are sex-linked factors affecting earlobe color, females from reciprocal F_1 crosses should differ in accordance with the effects of these factors in conjunction with those carried by the autosomes when in a heterozygous condition.

Matings 1 and 7 and 2 and 10 of table 1 afford data for comparison of the results of reciprocal F_1 crosses. For the first-mentioned matings the daughters of the white earlobe male show more white in the earlobes than those of the reciprocal mating in which the father had red earlobes. For the second case (Rhode Island Red by White Leghorn) there is only a very slight difference between the reciprocal crosses but such differen ce as exist are in the same direction as the other two reciprocals. If these differences may be taken as significant they would seem to indicate that some determiners for earlobe color are located in the Z-chromosome. It should be noted, however, that in the cross of Jersey Black Giant by White Leghorn the male offspring of reciprocal crosses differ in the same manner as do the females. Since all sons receive a Z-chromosome from each parent, the male offspring of reciprocal crosses should not differ with respect to the sex-linked factors. So from the F_1 generation no conclusions can be drawn regarding the existence of sex-linked factors affecting earlobe color. From the distribution of F_1 males it cannot be said that either color of earlobe is dominant. Most of the F_1 individuals have earlobes which are intermediate in color.

The results of various crosses of the White Leghorn and Jersey Black Giant are given in table 3 for the purpose of showing whether important

TABLE 3

Results of the various crosses of White Leghorns and Jersey Black Giants for the study of inheritance of earlobe color.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOM-INATELY WHITE	PREDOM-INATELY RED	RED
1 Giant ***RR*** *RR*	Leghorn ***WW*** *W*	***RW*** *RW*	***RW*** *R*	♀ 1 ♂ 2	20 17	27 24	2 1
2 Leghorn ***WW*** *WW*	Giant ***RR*** *R*	***RW*** *RW*	***RW*** *W*	♀12 ♂12	11 11	6 7	1
3 F_1 ***RW*** *RW*	F_1 (G♂×L♀) ***RW*** *R*	***RR*** / ***RW*** *RR*; ***WW*** *RW*	***RR*** / ***RW*** *R*; ***WW*** *W*	♀10 ♂ 2	27 28	17 36	1 10
4 F_1 ***RW*** *RW*	F_1 (L♂×G♀) ***RW*** *W*	***RR*** / ***RW*** *RW*; ***WW*** *WW*	***RR*** / ***RW*** *R*; ***WW*** *W*	♀16 ♂30	51 42	34 28	10 8
5 F_1 ***RW*** *RW*	Giant ***RR*** *R*	***RR*** / ***RW*** *RR*; *RW*	***RR*** / ***RW*** *R*; *W*	♀.. ♂..	14 3	54 29	28 56
6 F_1 ***RW*** *RW*	Leghorn ***WW*** *W*	***RW*** / ***WW*** *RW*; *WW*	***RW*** / ***WW*** *R*; *W*	♀17 ♂22	10 2	1 1	.. ..
7 Leghorn ***WW*** *WW*	F_1 (G♂×L♀) ***RW*** *R*	***RW*** / ***WW*** *RW*	***RW*** / ***WW*** *W*	♀31 ♂33	1 3	.. 3	.. ..

sex-linked factors are involved in the determination of earlobe color. Because of the variety of F_2 matings made in the cross this set of data is most satisfactory for analyzing the situation. In this table symbols are used to indicate the genetic constitution of each parent and the male and female offspring. The symbols in bold-face type indicate the origin of the two members of any pair of autosomes which may carry major factors influencing earlobe color. The symbols in italics show the origin of the Z-chromosome. The letter W in bold-face type signifies that the particular autosome was derived from the white earlobe parent while the same letter in italics marks a Z-chromosome originating in the white earlobe parent. The letter R is used in the same manner to indicate chromosomes originating in the red earlobe parent. Under the son and daughter headings are found listed all possible genotypes with reference to the sex chromosomes and any one pair of autosomes.

The histograms of figure 1 show graphically the distribution of earlobe color for both males and females of the last five matings of table 3. The solid line represents the distribution for the females and the dash line for the males. The four sections of each histogram represent the four grades of earlobe color; namely, white, predominately white, predominately red and red. The left hand section of each histogram is for white and the other

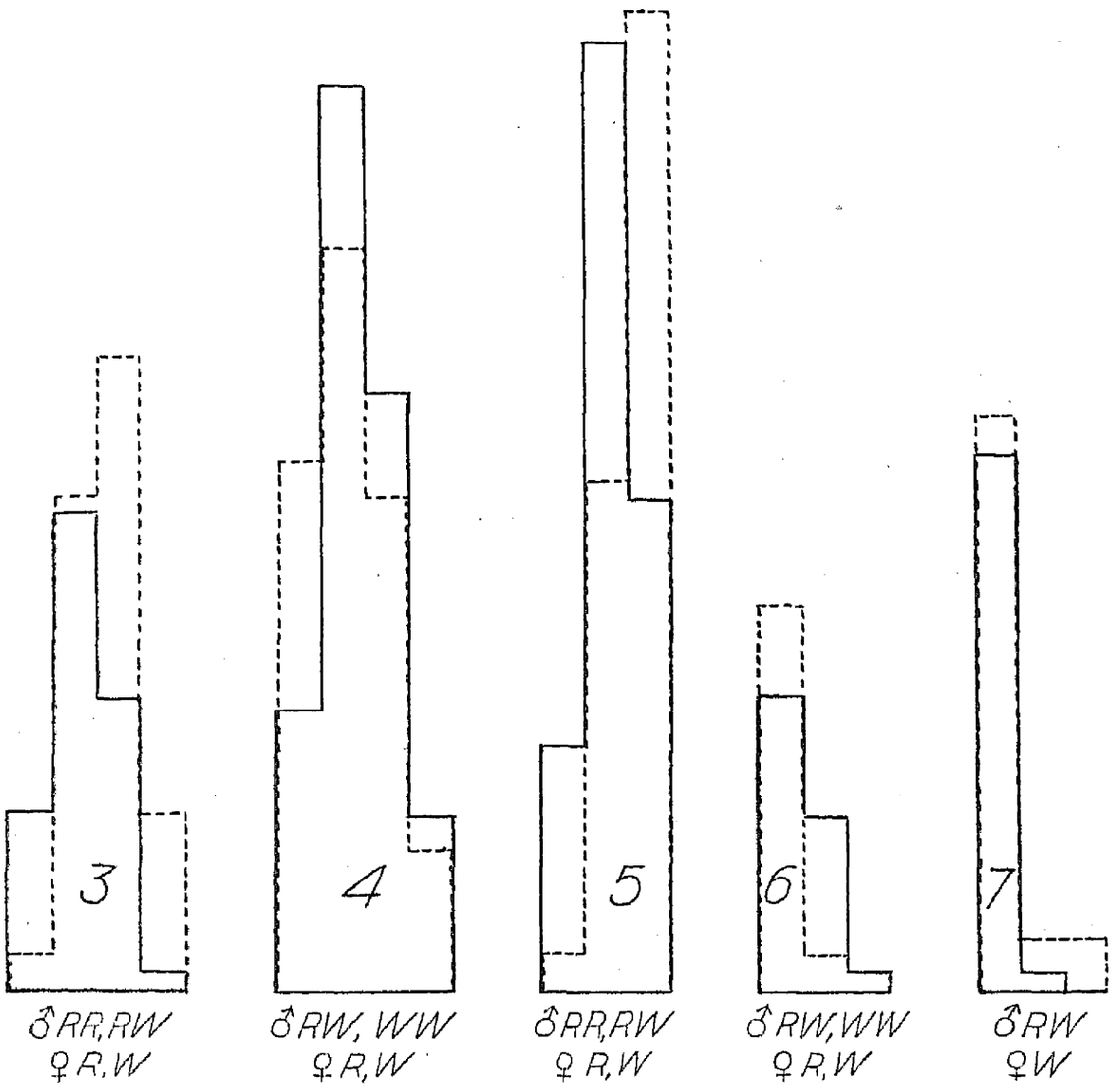


FIGURE 1.—The effect of sex-linked determiners upon earlobe color. The histograms present graphically a comparison of the distribution of earlobe color for males and females of the last five matings in table 3. The solid line indicates the distribution for females and the dash line for males. The letters below the graphs indicate the origin of the sex-chromosomes for the two sexes, *R* being for chromosomes originating in the red earlobe stock and *W* for those from the white earlobe stock.

grades follow in the order just given. Below each diagram is indicated the origin of the Z-chromosomes in the two sexes. In each mating, the genetic constitution of males and females with respect to autosomal genes is, of

course, the same. If differences between the two sexes are consistently associated with differences in origin of the sex chromosomes then it may be held that they are due to varying constitutions of the individuals from which the chromosomes came.

For all matings except the last it will be seen that the females are identical with respect to their Z-chromosomes, half receiving their only sex chromosome from the red and half from the white earlobe breed. In the histogram for mating 3 it will be seen that the earlobes of the males vary more in the red direction than do those of the females. Here half of the males are homozygous for sex chromosomes from the red earlobe stock while the other half are heterozygous. The two sexes are identical with respect to their autosomal constitution so if we assume that the diploid condition as found in the male is more effective than the haploid in the female, then the males should vary more in the red direction than do the females. In mating 4 it will be noted that the males are equally divided between those homozygous for the "white" chromosome and those heterozygous for the sex-linked factors. If the factors carried by the sex chromosome influence earlobe color, then it would be expected that the males would have whiter earlobes than those in the previous mating(since the autosomal constitution of the two matings are the same) and that they should be whiter than those of their sisters. An examination of histograms 3 and 4 will prove that both these conditions are met. The females of these two matings are identical with respect to both sex chromosomes and autosomes and their histograms also are quite similar.

The histograms 5 and 6 are for matings of the same designation in table 3. It will be seen that since in mating 5 the males are half homozygous for the "red" chromosome and half heterozygous they should have earlobes which are less white than their sisters. In mating 6, the formulas of their sex chromosomes would indicate that the males should have whiter earlobes than their sisters. The histograms for these two matings show the distribution of color of earlobes for the two sexes to be in agreement with their sex chromosomal composition.

The last histogram which represents the distribution of earlobe color for mating 7 shows practically no difference between the color of earlobes of the two sexes. It will be noted that the males are all heterozygous for any factors on the sex chromosome influencing earlobe color. This result is in agreement with the reciprocal F_1 matings. These two matings indicated in table 3 as 1 and 2 show the distribution of earlobe color to be quite similar for the two sexes.

The results in table 3 and figure 1 show fairly clearly that sex-linked factors are operative in influencing earlobe color. It would seem, however, that the determiners for earlobe color carried by the Z-chromosome are effective only when they are in a homozygous and diploid condition. It is true, at least, that in all matings where half of the male offspring were homozygous for genes of the red or white earlobe stock they differed from the females in earlobe color in the expected direction. In matings where the males were all heterozygous for sex-linked determiners there was no difference between the earlobe color of the males and females.

In crosses of other breeds the matings are not as complete as the one involving Jersey Black Giants and White Leghorns. The matings of Rhode Island Reds and White Leghorns shown in table 4 indicate that

TABLE 4

Results of crosses of White Leghorn by Rhode Island Red.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
Red *RRRR*	Leghorn *WWW*	*RWRW*	*RWR*	♀ . . ♂ 1	2 1	21 19	1 3
Leghorn *WWWW*	Red *RRR*	*RWRW*	*RWW*	♀ . . ♂ . .	9 . .	19 14	3 9
F_1 *RWRW*	F_1 (*Rd*♂ × *L*♀) *RWR*	*WW*, *WR* } *RW* *RR* } *RR*	*WW*, *WR* } *R* *RR* } *W*	♀ 5 ♂ 3	12 13	22 23	7 7
F_1 *RWRW*	F_1 (*L*♂ × *Rd*♀) *RWW*	*WW*, *WR* } *RW* *RR* } *WW*	*WW*, *WR* } *R* *RR* } *W*	♀ 2 ♂ 5	4 9	16 15	6 11

sex-linked factors are not operative. An examination of these matings shows that although the sons of the two F_2 matings differ in their sex chromosomes, their earlobe colors are quite similar and differ but little from those of their sisters.

In table 5 are shown the F_2 results from crosses of Brown Leghorn by Silver Penciled Rocks. Here the white earlobe is carried by the Brown Leghorns and the red by the Penciled Rock. A comparison of the distribution of earlobe color and the sex-chromosomes indicates slightly that sex-linked factors may be involved but evidence supporting this view is not found in all matings.

TABLE 5

Results of crosses of Brown Leghorns by Silver Penciled Rocks.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
F_1 *RWRW*	F_1(*L*♂×*Rd*♀) *RWW*	*WW*, *RW* } *RW*; *RR* } *WW*	*WW*, *RW* } *R*; *RR* } *W*	♀21 ♂32	26 26	14 5	1 1
F_1 *RWRW*	Br. Leghorn *WWW*	*RW* } *RW*; *WW* } *WW*	*RW* } *R*; *WW* } *W*	♀59 ♂59	4 1	2 ..	.. ..
F_1 *RWRW*	S. P. Rocks *RRR*	*RW* } *RW*; *RR* } *RR*	*RW* } *R*; *RR* } *W*	♀ 2 ♂ 1	2 1	4 2	1 4

Table 6 gives the data for the F_2 results of mating Buff Leghorn by

TABLE 6

Results of crosses of Buff Leghorn by White Wyandotte and Barred Plymouth Rock.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
WYANDOTTE×BUFF LEGHORN							
F_1 *RWRW*	F_1(*Wy*♂×*L*♀) *RWR*	*WW*, *RW* } *RW*; *RR* } *RR*	*WW*, *RW* } *R*; *RR* } *W*	♀85 ♂40	15 38	3 5	.. 3
F_1 *RWRW*	W. Wyandotte *RRR*	*RW* } *RW*; *RR* } *RR*	*RW* } *R*; *RR* } *W*	♀ 2 ♂	9 1	12 8	5 13
BARRED ROCK×BUFF LEGHORN							
F_1 *RWRW*	F_1(*Rk*♂×*L*♀) *RWR*	*WW*, *RW* } *RW*; *RR* } *RR*	*WW*, *RW* } *R*; *RR* } *W*	♀14 ♂12	15 12	20 11	15 9
F_1 *RWRW*	Buff Leghorns *WWW*	*WW* } *RW*; *RW* } *WW*	*WW*, *RW* } *R*; *RR* } *W*	♀42 ♂39	12 13	5 4	1 2

White Wyandotte and Buff Leghorn by Barred Plymouth Rock. In the former cross there is evidence that sex-linked factors are involved in bringing about the difference in earlobe color. The data shown for the Buff Leghorn-Barred Plymouth Rock cross, if checked for the correspondence in distribution of sex chromosomes and earlobe color, furnish no evidence for the view that sex-linked factors are here operative.

AUTOSOMAL FACTORS

The data already presented indicate that sex-linked factors are involved in determining earlobe color in some breeds. In some cases their influence appeared to be slight and in other crosses there was no evidence for sex-linkage of factors responsible for the existing differences in earlobe color. These results would lead one to expect evidence for autosomal factors for color of the earlobe. If so the data already presented in table 3 should afford such evidence. The columns giving the genotypes of the male and female offspring show some instances where the constitution with respect to the sex chromosome are identical but where differences in the autosomal constitution exist. The female offspring of matings 4 and 5 offer material for a comparison of this sort. Although their sex chromosomal constitution is the same we find a wide difference in their earlobe color. Associated with this difference in distribution of color of earlobes we see that there is a difference in the autosomal constitution of the two lots of females. Those in mating 4 have the three genotypes which result from mating two heterozygous individuals, one-fourth homozygous for white, one-half heterozygous, and one-fourth homozygous for red. In mating 5, half the females are homozygous for red and half heterozygous, none being homozygous for white. The great difference found here shows that the major factors involved are probably autosomal in location.

A still greater difference is shown by matings 5 and 6. Here the female offspring of the two matings have identical sex-chromosomal composition but differ considerably in the origin of their autosomes. In 5 half are homozygous for factors of the autosomes from the red earlobe parent while in 6 half are homozygous for factors from the white earlobe stock. In each case half of the offspring are also heterozygous. Since mating 6 produces no phenotypes homozygous for the R-factor we would expect it to differ from mating 5 to a greater degree than does mating 4. These relations are in agreement with differences in the factorial constitution of their autosomes. Matings 5 and 6 are reciprocal back crosses to the parent stocks and since in mating 6 a higher percentage of whites occur than reds in mating 5 there is evidence that at least some of the white autosomal factors are partially dominant to the red.

The evidence is fairly conclusive that at least one pair of autosomal factors is effective in determining earlobe color. A comparison of the results of matings 2 and 7 seems to indicate that more than one pair of autosomal factors are operative. The female offspring of the two matings have identical constitution with respect to the sex chromosomes. They

differ in that in mating 2 all are heterozygous for autosomal factors while in mating 7 half are of this constitution and half are homozygous for any given pair of autosomes from the white earlobe parent. If only one pair of autosomes are involved in determining earlobe color, then half of the female offspring in mating 7 should have earlobes the color of which show a distribution similar to that in mating 2. The data show that practically all of the females in mating 7 have white earlobes. Most of the expected intermediates are missing. This shortage of intermediates might be expected if there exists another autosomal factor which segregates independently of the first. If either autosomal factor in a homozygous condition is capable of producing birds with white earlobes but few earlobes other than white would be expected. It thus seems probably that more than one pair of autosomal factors are operative.

LINKAGE OF SEX-LINKED DETERMINERS

Since it has been shown that both autosomal and sex-linked factors are involved in determining earlobe color it is of interest to know whether earlobe color shows linkage with other known characters. The fact that some of its determiners show sex-linked behavior suggests that earlobe color should show linkage with some member of the sex-linked group. Since something is known of the distribution of the sex-linked factors it is possible to determine with some degree of accuracy the relative position of at least the major sex-linked factors influencing earlobe color. The writer (Anatomical Record **29**: 143–144) has shown that the factors for the sex-linked character determining rate of feathering is widely separated on the sex chromosome from those for barring and shank color. Hence, a cross involving these characters and differences in earlobe color should show in which end of the sex chromosome the sex-linked factor for earlobe color lies.

In table 7 are shown the linkage relations of earlobe color with rate of feathering and shank color, and rate of feathering and barring. These data are from the F_2 generation of crosses of the Jersey Black Giant and White Leghorn. In the table are included only the crossover classes, first for rate of feathering and shank color, and then rate of feathering and barring. Since the original combinations were rapid feathering and yellow shanks, and slow feathering and dark shanks, the crossover classes in the first section of the table are slow feathering-yellow and rapid feathering-dark. In the original crosses white earlobe entered with yellow shanks and rapid feathering. Since the determiners for yellow and rapid have been shown to occupy distant sections of the sex chromosome, the crossover

classes in table 7, representing breaks in the sex chromosome between these points should indicate the position of the sex-linked determiners for earlobe color in relation to these two factors. When the determiners for yellow and rapid separate in crossing over, the determiners for white earlobe will go most frequently with the point with which it is most closely associated. Thus, since the original combination was yellow shank and rapid feathering, the two crossover classes will include each character combined with the allelomorph of the other and the group having the whiter earlobes will show which of these two points the earlobe color factor lies nearer.

Figure 2A shows the probable relation upon the sex chromosome of factors for rate of feathering and shank color. The diagrams 2B and 2C show the sex chromosome following the phenomenon of crossing over,

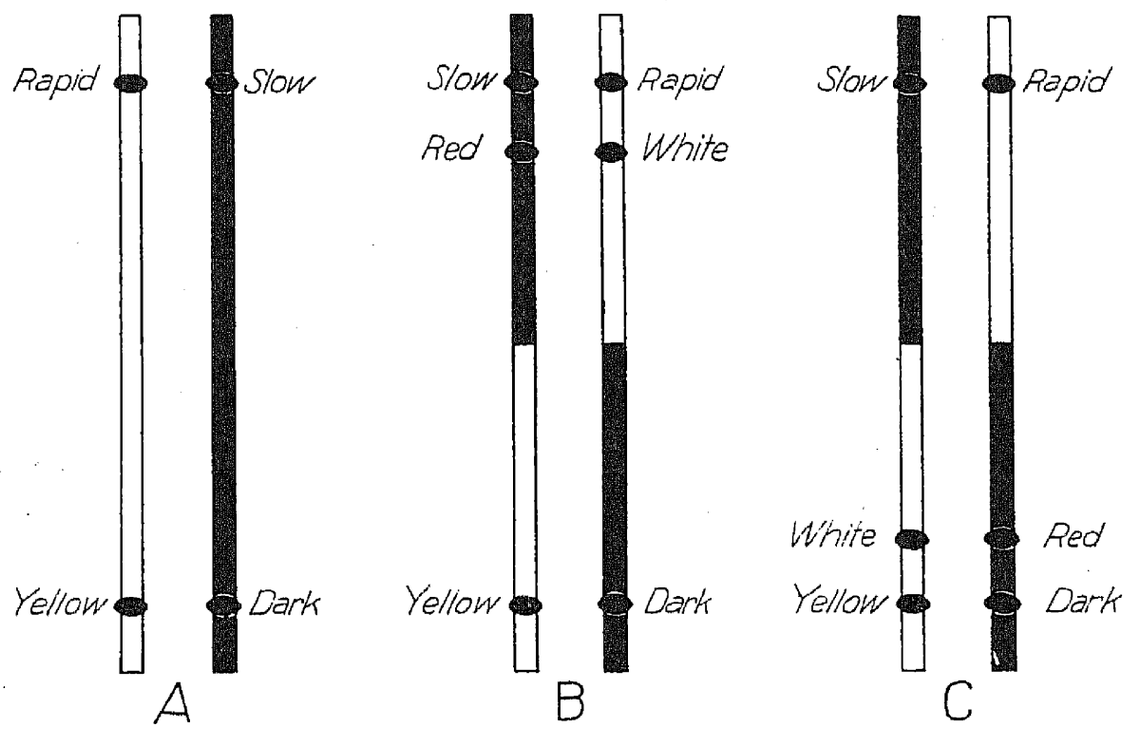


FIGURE 2.—Diagrams showing the various crossover combinations depending upon which end of the chromosome the sex-linked factor for earlobe color is located. Figure 2A gives the probable location of the factors for rate of feathering and shank color. Figure 2B gives the most frequent crossover combinations when the earlobe color factor is nearer the gene for rate of feathering while figure 2C shows the situation where it is located nearer the gene for shank color. In table 7 are found the data for these linkage relations.

B indicating the most frequent grouping of characters providing the gene influencing earlobe color is in the same end with that for rate of feathering. The situation when the determiner for earlobe color is in the opposite end is shown in C. If the situation shown in B be the true one,

then the crossover group rapid-dark should be whiter than its reciprocal slow-yellow. If the diagram C represents the true relation then the conditions stated above would be reversed, the crossover group slow-yellow being the whiter.

Table 7 shows the crossover group slow-yellow to be whiter than its reciprocal and hence substantiates the view that the sex-linked determiner

TABLE 7

Linkage relations of sex-linked factors for earlobe color.

Crossover group	Earlobe color			
	White	Predominately white	Predominately red	Red
RELATION TO RATE OF FEATHERING AND SHANK COLOR				
Rapid dark	4	17	13	5
Slow yellow	16	22	11	4
RELATION TO RATE OF FEATHERING AND BARRING				
Rapid non-barred		4	9	4
Slow barred	1	3	3	3

for earlobe color is located in the chromosome nearer the shank color determiner than that for rate of feathering.

It has also been shown (WARREN 1924) that the determiner for barring is also distantly separated from the rate of feathering gene and probably located near the gene for shank color. If this be true then earlobe color should show relations to this factor similar to those found for shank color. The original combination was white earlobe, rapid feathering, barred plumage so the barred-slow crossover group should be whiter than the group rapid-non-barred. There is a slight difference in distribution in the expected direction. Since the numbers involved are small these results cannot be held to be conclusive but may be taken as a suggestion of the possible relations. Also, since the effects of the sex-linked determiners have been shown to be slight in the presence of the autosomal factors, large differences could not be expected in the data shown in table 7.

LINKAGE OF AUTOSOMAL FACTORS

It is also possible to determine whether linkage relations exist between any autosomal earlobe color factors and factors for certain known autosomal characters. Several autosomal characters were involved in the

crosses, but for only two have the linkage been measured since in most instances the characters were not sufficiently clean-cut in their segregation to permit accurate classification. No autosomal linkage groups have yet been determined in poultry so it is not known whether the characters used belong to the same or different linkage groups. Although dominant white and extended black plumage colors have been treated as an allelomorphic pair the evidence substantiating this assumption is not entirely conclusive. This fact should not however influence the results.

Any character, the factor for which lies in the same chromosome with autosomal earlobe factors, should in segregation more frequently be associated with the earlobe color with which it originally entered. Thus in a cross involving white and black plumage and red and white earl obes, since white plumage and white earlobe entered the cross together, it is expected that in the F_2 generation the white plumage segregates will have the whiter earlobes, providing the factors for these two characters are in the same chromosome. The segregation of rose and single comb should in the same manner indicate their linkage relations with earlobe color. If no linkage exists the distribution of earlobe color among the classes of segregates should be a random one. Table 8 shows the distribution of

TABLE 8

Linkage relations of autosomal factors for earlobe color

CONTRASTED PAIR	EARLOBE COLOR			
	White	Predominately white	Predominately red	Red
White		9	46	44
Black		8	35	35
Rose	67	56	16	2
Single	29	21	9	1

earlobe color for F_2 segregates of black and white plumage and rose and single comb. It will be noted that the distribution of earlobe color for the two members of each group is practically identical, thus indicating that the determiners for these characters are not carried by any chromosomes bearing the major autosomal determiners for earlobe color.

LINKAGE RELATIONS OF EARLOBE COLOR AND EGG COLOR

In the established breeds and varieties of poultry white egg shell color has usually been associated with white earlobe color, while the red earlobe breeds usually lay eggs of the darker shades. This has led practical breeders

to hold that there is some relation between earlobe and egg color. The apparent linkage might be due to close association of the determining genes on the chromosome or to the existence of a single factor which controls the pigmentation of both the egg and earlobe. If the existing association between earlobe and egg color is a chance one, the linkage should be broken by segregation.

To determine the nature of the association between these two characters 62 F_2 females chosen at random from the cross Jersey Black Giant by White Leghorn were tested for egg color. For classifying (the egg colors) a color chart was made grading from white to the darkest brown shade. The color grade was determined by taking the average color of five eggs laid during the first month of production. Other studies have shown that egg color varies somewhat with the period of production and for that reason the selection was limited to the first month.

In table 9 is shown the distribution of egg color for the 62 females,

TABLE 9

Showing independent assortment of factors for earlobe color and egg shell color.

EARLOBE COLOR	EGG COLOR GRADES								
	1	2	3	4	5	6	7	8	9
White	..	..	1	1	4	..	1	1	..
Predominately white	2	2	2	5	8	5	1	..	1
Predominately red	..	1	4	4	6	4	2	..	..
Red	2	1	..	1	2	1	1	..	..

grouped according to earlobe color. Grade 1 is white and the succeeding grades become progressively darker. It is seen that individuals falling into the four grades of earlobe color lay eggs of practically the same average color and hence there is no evidence for any conditions which tend to associate white earlobe with white egg color. This would seem to show that the existing association is entirely a chance one. It is possible that this association may bear some relation to the origin of breeds of poultry.

GENERAL DISCUSSION

The experiments discussed indicate that earlobe color is dependent upon several genetic factors. The fact that all grades of earlobe color are found in the F_1 generation from crossing most white and red earlobe breeds would lead one to expect a complex situation.

It also seems probable that individuals of a single strain of a breed may differ in some of the minor factors influencing earlobe color. The results of the F_2 generation indicated that individuals of some breeds were not homozygous for all factors for earlobe color. Greater differences in factorial constitution probably exist between breeds. Crosses of the Rhode Island Red breed showed no evidence of its carrying sex-linked earlobe color determiners while crosses of other breeds indicated genes of this chromosome to be operative. The differences in genetic constitution of individuals of a breed do not seem to affect the major earlobe color factors since the breeds dealt with seldom produced individuals varying from the standard.

From crosses of the Single Comb White Leghorn and Jersey Black Giant breeds there is evidence for the existence of at least three factors influencing earlobe color. A sex-linked factor is operative but seems to play a minor role since its influence was slight and exhibited only when in a homozygous diploid condition and acting with some of the autosomal factors in a heterozygous condition. The major earlobe color factors are autosomal and there is evidence that at least two factors of this group are operative. This complex situation is probably only the natural result of the manner in which the color has been fixed. Since the slightest variation from either white or red constitute a breed disqualification any modifying factor which tended to maintain the constancy of the desired color was seized upon and by selection incorporated in the genetic constitution of the breed.

SUMMARY

1. Earlobe color has a complex factorial basis.

2. Breeds having the same earlobe color may differ considerably in their genetic constitution with respect to this character.

3. Individuals of a single breed and strain may differ in some of the minor genetic determiners.

4. The Jersey Black Giant and White Leghorn breeds differ in at least three factors determining earlobe color, one sex-linked and two autosomal.

5. It was possible to estimate roughly the position upon the sex chromosome of the sex-linked factor.

6. The autosomal factors for earlobe color showed no linkage with those for any autosomal characters which the crosses permitted testing.

7. There was no evidence of any linkage between the factors for earlobe color and egg color.

A NEW SEX-RATIO ABNORMALITY IN *DROSOPHILA OBSCURA**

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Received February 27, 1928

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MATERIALS AND METHODS

Drosophila obscura Fallén is commonly found in the neighbourhood of Moscow. Apparently, it in no way differs from the American fly; at least, the coincidence between all the morphological characters of both species is complete. The cytological investigation of the Russian *D. obscura* is unfinished as yet; some preliminary data, which S. L. FROLOVA most obligingly communicated to me, mark the presence of a long V-shaped X-chromosome, perfectly distinct from the rodlike Y-chromosome. The American *D. obscura*, according to METZ and MOSES (1923), has a bent X-chromosome which, as in the Russian species, differs distinctly from the straight and shorter Y-chromosome.

In America *D. obscura* is usually raised upon bananas; in our laboratory, in spite of many experiments, no completely satisfactory diet has yet been found. The best results are obtained with a nutritive medium prepared from a mixture of fermented raisins (100 g.), potatoes (400 g.), agar-agar (4 g.) and water (800 ccm.), but even on this medium the percent of perishing cultures is comparatively large. Also the number of offspring produced by one pair is less than that usually observed in other species of Drosophila. I could never succeed in obtaining a mating that produced more than 200 flies, and the mean number for the offspring in a culture did not rise above 40–45.

* The GALTON AND MENDEL MEMORIAL FUND pays part of the cost of the accompanying tables.

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The flies were raised in sterilized flat-bottomed glass cylinders, containing some nutritive medium lightly sprinkled with yeast; the cylinders were corked by thick cotton-wool stoppers. The cultures were kept in a temperature oscillating from 21° to 25°C. Particular attention was paid to the virginity of the females used for mating; only such were considered virgin as had yet folded wings, or had been hatched in the absence of males (for instance from isolated pupae). On an average, the development of the fly took about 20 days, but this term varied considerably, chiefly in accordance with the temperature. The flies hatched were narcotized with ether and carefully examined through a binocular microscope, all the results being recorded in detail.

TABLE 1

OFFSPRING MORE THAN 20 FLIES

NUMBER OF CULTURES	NUMBER	♂♂	♀♀	TOTAL	PERCENT ♂♂
	1	21	23	44	47.7
	3	14	16	30	46.7
	4	13	13	26	50.0
	6	26	26	52	50.0
	8	22	18	40	55.0
	9	23	19	42	54.8
	10	20	14	34	58.8
	11	21	21	42	50.0
	12	21	19	40	52.5
	13	10	10	20	50.0
	14	18	25	43	47.9
	15	17	18	35	48.6
	16	14	10	24	58.3
	18	17	15	32	53.1
	19	35	33	68	51.5
	20	13	19	32	40.6
16	..	305	299	604	50.4

OFFSPRING LESS THAN 20 FLIES

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PERCENT ♂♂
3	..	20	28	48	41.7

TOTAL

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PERCENT ♂♂
19	..	325	327	652	49.9

APPEARANCE OF THE ABNORMALITY

In July, 1925, I caught in a forest near the Hydrophysiological Station in Zvenigorod (near Moscow) 19 females of *D. obscura*. They were all fecundated already before being caught, and the offspring they gave were perfectly normal as regards the sex-ratio (table 1). Altogether 327 females and 325 males were obtained, thus giving a relation of 1.003:0.997, instead of the expected 1:1. From each culture of the first generation ten pairs of flies were inbred. In the F_2, both in the sum total and in the separate crosses the offspring of 17 of the wild females showed a normal sex-ratio. At the same time in the progeny of two females a sharp deviation from the expected relations was found. The appearance of these abnormalities is shown in figure 1. In two lines, line 3 and line 13, cultures

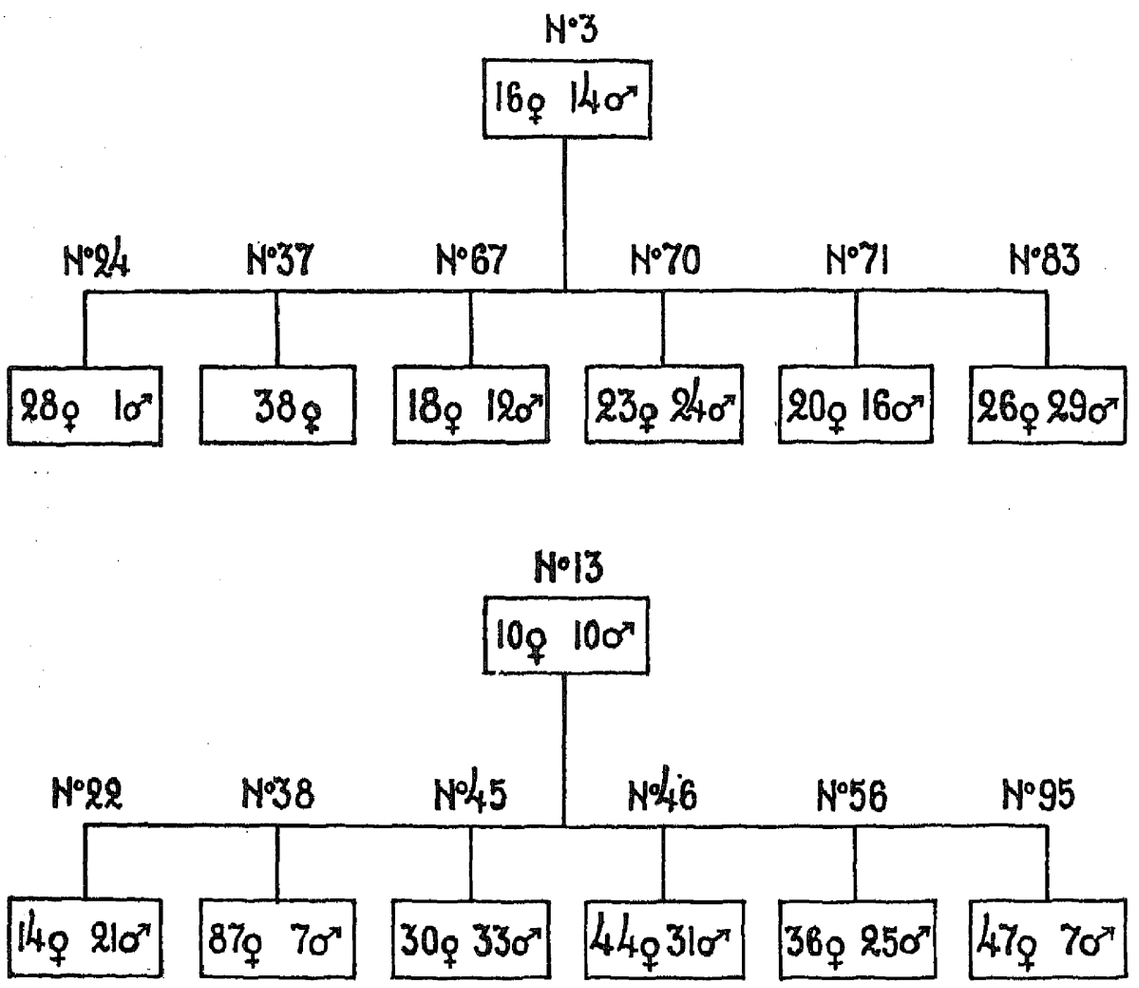


Figure 1

appeared totally or almost totally consisting of females. Altogether, four such cultures were obtained (Nos. 24 and 37 in line III and Nos. 38 and 95 in line XIII). The deviations from the normal sex-ratio were so considerable that it seemed impossible to explain them by accidental causes. A further analysis of the phenomena was therefore undertaken.

CHARACTERISTICS OF THE ABNORMALITY

The females resulting from the abnormal cultures of the second generation of lines 3 and 13 were mated in both lines partly with their brothers, partly with males from kindred normal cultures. The first generation proceeding from these matings showed itself everywhere perfectly normal. Further, in the succeeding generations, the offspring of these flies were bred in both lines by close inbreeding as well as more freely, within the limits of the line. A part of these crosses always showed a normal sex-ratio, while the other gave a progeny resembling, as to its results, the original abnormal cultures, namely, either consisting exclusively of females, or presenting only an insignificant number of males. Both in lines 3 and 13 the number of males in these cultures varied between 0 and 15 percent, on an average however not exceeding 4 to 5 percent of the total number of flies. Henceforth cultures showing such a predominance of females will be spoken of as "female" ones.

For several consecutive generations no more detailed investigation of the described abnormality was undertaken. During all this period I confined myself to maintaining line 3 by crossing the females from the "female" cultures either with their brothers, if such existed, or else with males from normal cultures of the same line. Also the progeny of these normal cultures was occasionally inbred. In all these cases "female" cultures were obtained as well as normal ones. As a result of all these crossings I was able to collect somewhat extensive statistical materials, which showed in line 3 of *D. obscura* the presence of but two types of sex-ratios, the normal and the "female" one, and the total lack of any transitions between them. The results are represented in figure 2. They include all the individual matings, in which the flies of line 3 participated, and which resulted in more than 20 offspring. All the matings are divided, according to the percent of males in the offspring, into classes, which are shown on the abscissa. The number of matings belonging to each class are shown on the ordinates. As can be easily seen, all the crosses fall into two independent groups. The first one includes the cultures with a practically normal sex-ratio, that is, with about 50 percent of males. This group constitutes the right curve of the distribution, which is almost regular and shows that the observed slight deviations from the expected relations are due here to accidental causes. All the other cultures form the left curve; the average number of males is here only 4.1 percent of the total number of flies and the mode of the curve lies at zero. No intermediate cultures appeared which could mar this picture.

This table can be compared with another one, constructed exactly on the same plan for the seventeen normal lines of *D. obscura* (figure 3). Figure 3 includes all the individual matings from all the normal lines, that is, from all the inbreeding lines originating from the wild females, except 3 and 13. The distribution of the cultures is here quite analogous

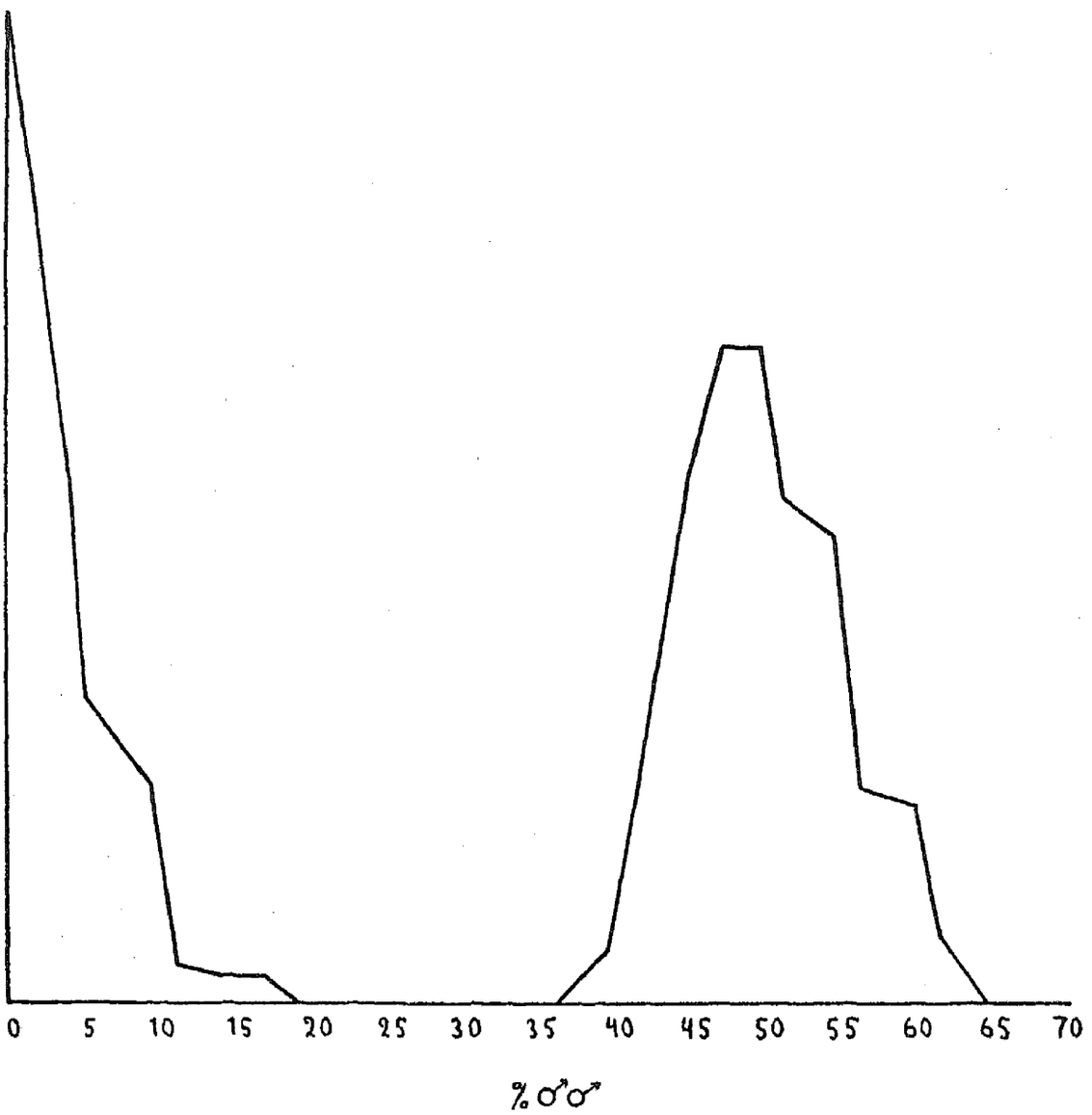


FIGURE 2.—Left curve: M=4.1; n=102. Right curve: M=49.0; n=185.

to that presented by the right curve of figure 2; no "female" or other deviating sex-ratios are met with.

Altogether, the preliminary results of this work can be summed up as follows. The researches upon the seventeen lines, started from wild females, have made it clear that as a rule the deviations from the normal sex-ratio are in *D. obscura* very small and can be easily explained by accidental causes. The crosses in line 3 present at the same time some cultures with abnormal sex relations, the number of males being in them,

on an average, only about 4 percent of the total number of flies. No transitions are observed in this line between the normal and the deviating cultures. An analogous phenomenon was observed in another line (13) while it existed.

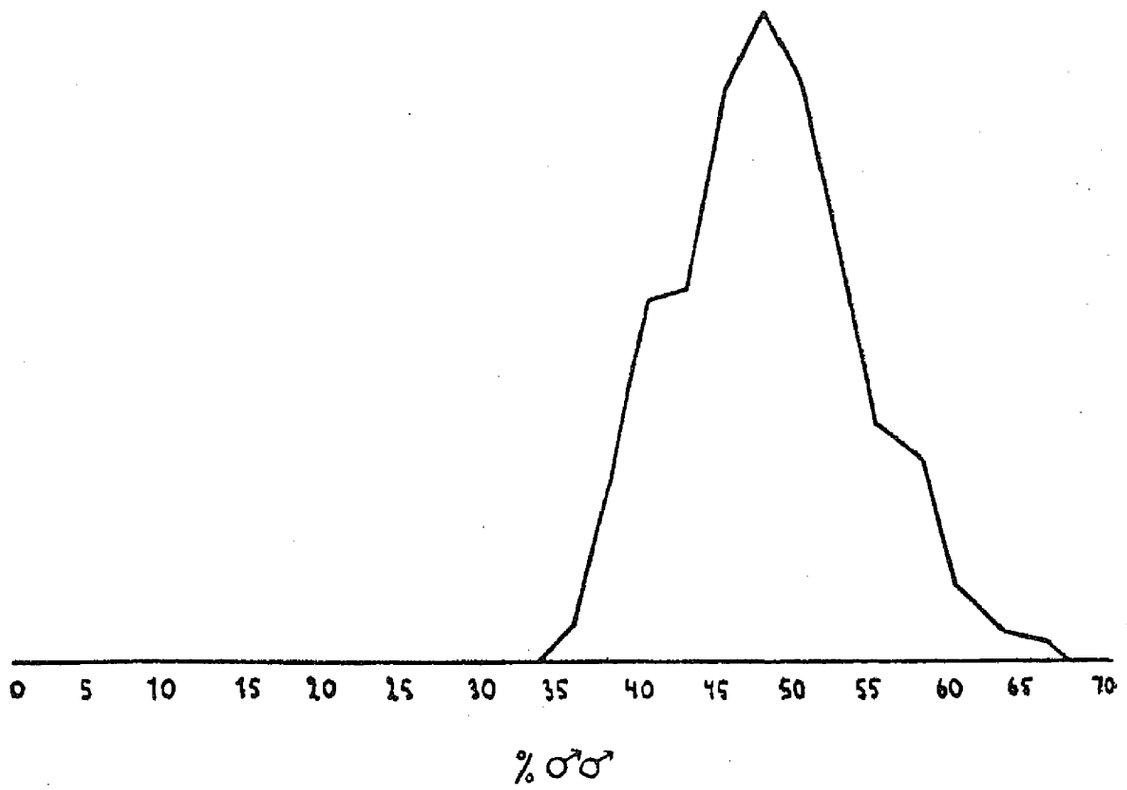


FIGURE 3.—M = 48.0; n = 194.

The data enumerated show that this abnormality is certainly hereditary, but, on the basis of these preliminary investigations it has not been possible to give its more detailed genetical characteristics; in order to do so, a whole new series of special crossings had to be organized.

GENETIC ANALYSIS OF THE ABNORMALITY

By mating a female from the "female" culture No. 1500 with its only brother, an offspring was obtained with a normal sex-ratio (49.4 percent ♂♂) and a comparatively large number of flies. This culture received the No. 1644 and served as a basis for all the subsequent investigations. Two series of crosses were organized from its offspring. The first series, designated as "type I," was produced by individual matings of virgin females from the culture No. 1644 with males of a practically pure normal line of *D. obscura*, that had passed through more than ten generations of close inbreeding without ever presenting any deviations from the usual

sex proportions. All these crosses showed normal sex relations very near 1:1; there were altogether 17 crosses giving more than 20 flies and 5 crosses with a smaller number of offspring. These results are represented in table 2. The second series (type II) consisted of crosses, the reverse of the

TABLE 2

OFFSPRING MORE THAN 20 FLIES

NUMBER OF CULTURES	NUMBER	♂♂	♀♀	TOTAL	PER CENT ♂♂
	1734	10	12	22	45.5
	1735	23	16	39	59.0
	1738	17	15	32	53.1
	1742	21	21	42	50.0
	1744	21	14	35	60.0
	1746	26	35	61	42.6
	1751	22	22	44	50.0
	1752	10	11	21	47.6
	1755	21	26	47	44.7
	1758	18	12	30	60.0
	1761	21	23	44	47.7
	1763	17	15	32	53.1
	1765	20	25	45	44.4
	1766	14	19	33	42.4
	1768	24	28	52	46.2
	1770	35	32	67	52.2
	1771	18	15	33	54.5
17	..	338	341	679	49.8

OFFSPRING LESS THAN 20 FLIES

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PER CENT ♂♂
5	..	19	28	47	40.4

TOTAL

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PER CENT ♂♂
22	..	357	369	726	49.2

preceding ones; that is, virgin females from the pure normal line were crossed with males from No. 1644. Without any exception the crosses of this type showed "female" sex-ratios; 27 crosses were obtained, 19 of which gave more than 20 flies (table 3).

TABLE 3

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1673	4	41	45	8.9
	1674	..	24	24	0.0
	1677	2	19	21	9.5
	1680	3	42	45	6.7
	1682	1	32	33	3.0
	1683	1	25	26	3.8
	1685	..	25	25	0.0
	1687	..	34	34	0.0
	1689	..	29	29	0.0
	1690	4	33	37	10.8
	1693	1	26	27	3.7
	1710	2	37	39	5.1
	1713	3	71	74	4.1
	1715	..	51	51	0.0
	1721	..	22	22	0.0
	1726	..	28	28	0.0
	1729	2	28	30	6.7
	1731	..	20	20	0.0
18	..	23	587	610	3.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
9	..	2	97	99	2.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
27	..	25	684	709	3.5

The offspring of the crosses of type I did not undergo any further analysis. As to type II, two series of crosses were again started from its cultures. One series, named type III, consisted of crosses between virgin females from culture No. 1713 (of type II) and males from the pure normal line. All these crosses gave normal offspring; table 4 presents the numbers obtained. The other group included crosses between normal virgin females and the males out of all those cultures of type II, where they existed in the

TABLE 4

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1820	20	22	42	47.6
	1821	21	16	37	56.8
	1823	30	21	51	58.8
	1826	14	20	34	41.2
	1827	25	24	49	51.0
	1828	48	51	99	48.5
	1829	14	15	29	48.3
	1835	21	25	46	45.2
	1838	15	23	38	39.5
	1840	18	18	36	50.0
	1841	14	17	31	45.2
	1845	26	26	52	50.0
	1846	18	19	37	48.6
	1851	43	52	95	45.3
	1853	16	20	36	44.4
	1854	19	20	39	48.7
	1856	19	16	35	54.3
	1859	21	28	49	42.9
18	..	402	433	835	48.1

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
6	..	25	28	53	47.2

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
24	..	427	461	888	48.1

offspring. Nineteen such crosses (named type IV) were obtained, all of them normal in regard to the sex-ratio (table 5). Lastly, from the offspring of the culture of type III two more types of crossings were organized: the crossings of virgin females from the culture No. 1828 with males of the normal line (type V), and reversed crossings of normal females with males from the cultures of type III (type VI). All the matings of the V type presented normal sex-relations (table 6). On the contrary, in type VI "female" cultures again appeared.

TABLE 5

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1861	14	16	30	46.7
	1865	15	11	26	57.7
	1866	20	25	45	44.4
	1871	24	27	51	47.1
	1873	20	23	43	46.5
	1875	16	22	38	42.1
	1876	12	12	24	50.0
	1880	13	17	30	43.3
	1882	18	18	36	50.0
	1883	34	28	62	54.8
	1885	10	11	21	47.6
	1887	12	10	22	54.5
	1888	29	20	49	59.2
	1890	26	19	45	57.8
	1896	13	14	27	48.1
15	..	276	273	549	50.3

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
4	..	16	25	41	39.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
19	..	292	298	590	49.5

The crosses of type VI, though alike in structure, fall into two groups owing to the origin of the participating males: 27 cultures were obtained from the mating of males from the culture No. 1828 with normal virgin females, while 37 cultures resulted from analogous matings of males from culture No. 1851 (also of type III). Both in the first and in the second group "female" cultures appeared, and in both cases very nearly one half of the matings gave a "female" progeny (table 7 and table 8). Altogether 47 matings were obtained (leaving out those which produced less than 20 flies): 23 matings gave a normal offspring, and 24 matings presented typically "female" sex-ratios. Thus the relation between the number of normal matings and of "female" ones was found to be in type VI very near to 1:1 (table 9).

TABLE 6

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2021	10	15	25	40.0
	2023	26	21	47	55.3
	2027	24	17	41	58.5
	2028	19	17	36	52.8
	2032	16	12	28	57.1
	2035	24	30	54	44.4
	2036	11	9	20	55.0
	2037	14	13	27	51.9
	2045	13	12	25	52.0
	2048	19	22	41	46.3
	2051	18	24	42	42.9
	2053	16	13	29	55.2
	2054	18	19	37	48.6
	2060	14	17	31	45.2
	2061	26	26	52	50.0
	2065	10	12	22	45.5
	2066	26	22	48	54.2
17	..	304	301	605	50.2

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
6	..	28	35	63	44.4

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
23	..	332	336	668	49.7

This is the last series of matings undertaken in order to analyse the phenomenon of irregular sex-distribution in *D. obscura*. The examination of the results obtained allows us to draw some important conclusions as to the mode of inheritance of this abnormality.

First of all, wherever the females originate the progeny is perfectly normal, provided they are mated with males belonging to the pure normal line. This is distinctly to be seen in the matings of type I, III and V. Meanwhile, the matings of type II, arranged on the reverse principle,

TABLE 7

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2070	..	34	34	0.0
	2072	23	20	43	53.5
	2073	..	22	22	0.0
	2078	1	41	42	2.4
	2082	19	19	38	50.0
	2084	20	15	35	57.1
	2085	21	28	49	42.9
	2086	12	12	24	50.0
	2089	..	28	28	0.0
	2097	6	74	80	7.5
	2099	3	50	53	5.7
	2102	24	21	45	53.3
	2103	..	43	43	0.0
	2105	26	30	56	46.4
	2106	2	31	33	6.1
	2110	1	31	32	3.1
	2112	26	25	51	51.0
	2117	14	19	33	42.4
	2122	1	19	20	5.0
	2123	30	32	62	48.4
20	..	229	594	823	27.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
7	..	25	79	104	24.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
27	..	254	673	927	27.4

where the females were taken from the acknowledged normal line, and the males alone belonged to line III, yielded all a clearly expressed "female" offspring; the same was observed for half of the matings of type VI. This indicates that all the males from No. 1644 and half of those from cultures of type III, when mated even with pure normal females, possess the faculty of giving even in the first generation the same deviating sex-ratios as characterize line 3 of *D. obscura*.

TABLE 8

OFFSPRING MORE THAN 20 FLIES

Number of cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2125	14	14	28	50.0
	2129	4	69	73	5.5
	2131	1	34	35	2.9
	2135	18	20	38	47.3
	2136	..	36	36	0.0
	2138	23	23	46	50.0
	2145	17	11	28	60.7
	2147	1	21	22	4.5
	2148	..	30	30	0.0
	2162	..	44	44	0.0
	2164	20	24	44	45.5
	2165	24	23	47	51.1
	2166	2	41	43	4.7
	2167	..	40	40	0.0
	2174	29	23	52	55.8
	2175	11	13	24	45.8
	2178	24	26	50	48.0
	2179	1	39	40	2.5
	2180	..	20	20	0.0
	2183	2	19	21	9.5
	2184	24	22	46	52.2
	2186	17	16	33	51.5
	2191	..	35	35	0.0
	2192	18	24	42	42.9
	2903	..	51	51	0.0
25	..	250	718	968	25.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
7	..	22	88	110	20.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
32	..	272	806	1078	25.2

Thus, it can be considered as established that if the male descends from the pure normal line, the progeny will always be normal whatever may be the genetical structure of the female. On the contrary, males, directly

TABLE 9

SERIES	NUMBER OF NORMAL CULTURES	NUMBER OF "FEMALE" CULTURES	NUMBER OF CULTURES WITH OFFSPRING LESS THAN 20 FLIES
♂ from No. 1828 × ♀ from normal line	10	10	7
♂ from No. 1851 × ♀ from normal line	12	13	7
Total	22	23	14

descending from line 3, give a "female" offspring even when mated with pure normal females. Hence the inevitable conclusion, that in the present case the character of the numerical sex-relations is entirely determined by the genetic structure of the father. Whether the offspring will be normal or "female" depends only on the male taken for mating; the female does not play any part here. This was confirmed afterwards by matings of numerous males to each of several different females; the results obtained showed once more that the sex-ratio of the offspring is determined by the structure of the father only.

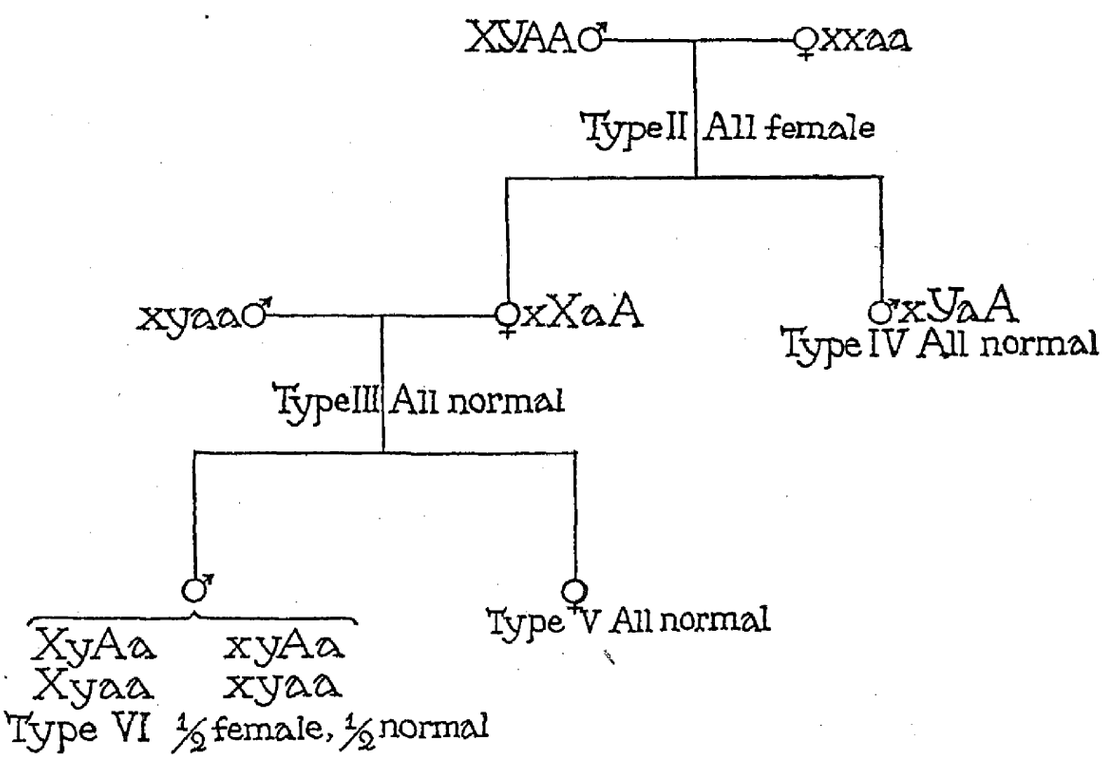


Figure 4

To explain the inheritance of the observed phenomenon by a violent alteration in the chromosomal apparatus, like non-disjunction, attachment of chromosomes, translocation or polyploidy, appears to be utterly impossible. Neither can plasmatic heredity explain the results of the crosses described. If we turn to the normal chromosomal mechanism, three hypotheses are possible. The factor, determining the abnormality studied, can be transmitted either by the Y or by the X-chromosome, or lastly by one of the autosomes. Figure 4 represents the inheritance of the sex chromosomes and the autosomes proceeding from line 3 (that is, those chromosomes capable of carrying the factor for the abnormality) throughout all the crosses described. For the sake of clearness, only one pair of autosomes is figured in the drawing. The chromosomes from line 3 are indicated by capitals.

It is easy to show that the Y-chromosome is not concerned in the transmission of this factor. The males that carry the Y-chromosome that comes directly out of line 3 nevertheless give a perfectly normal progeny (in type IV). On the other hand, males with a Y-chromosome originating from the pure normal line are capable of giving "female" cultures (type VI). This gives sufficient evidence that the Y-chromosome, whatever may be its origin, plays no part in the hereditary transmission of the gene calling forth the abnormality.

Examining the inheritance of the autosomes from line 3 we see that during the crosses described they could never attain a homozygous state. Consequently, if the factor which determines the appearance of the "female" ratios is transmitted by means of an autosome, it must be capable of acting in a heterozygous state; only thus could the appearance of abnormal sex relations in the crosses of type VI be explained. All the males from No. 1644 gave a "female" progeny; therefore they all carried the gene determining the abnormality. Even if we suppose them to have been only heterozygous for this gene, nevertheless at least half of their sons ought to have inherited it, in case it were really localized in one of the autosomes. However, their sons show in their offspring exclusively normal sex-ratios (in type IV). Therefore the supposition that the gene which causes the appearance of "female" culture lies in an autosome, must be rejected.

There remains the possibility of its being localized in the X-chromosome. For a verification of this, the males obtained from the cultures of type III must be examined. Half of these males carry the X-chromosome received from line 3; likewise, just the same quantity of males from type III have given "female" offspring. Were it possible to prove that the "female"

culture were obtained exactly from those males, which carried the X from line 3, this would prove the correctness of the supposition that the gene determining the abnormality is transmitted by the X-chromosome.

The remaining males from the cultures of type III bear the X-chromosome proceeding from the normal line. These males, according to their genotypical structure, must be subdivided into two groups. The males of one group do not in any wise differ from the males of the normal line: all their chromosomes are perfectly normal. It is evident that this group could not yield "female" cultures. The males of the other group have only the autosomes originating from line 3. As was already proved, the autosomes do not contain the gene producing the abnormality; hence these males likewise cannot give an offspring with the "female" sex-ratio.

Thus, only such males can produce a "female" progeny, as derive the X-chromosome from line 3 of *D. obscura*, and this includes all of them, because they quantitatively exactly correspond to the "female" cultures of type VI: half the cultures of type VI gave a "female" offspring, and half of the males from type III carry an X-chromosome from line 3. In this way, the supposition that the gene which determines the capacity of males to produce a "female" progeny is really localized in the X-chromosome, can be considered as established.

ACTION OF THE GENE PRODUCING THE ABNORMALITY

Analysis by hybridization has led to the conclusion that the factor which calls forth the appearance of "female" cultures is transmitted by the X-chromosome as an ordinary sex-linked gene. Turning to the action of this gene we see that, first of all, the males carrying it produce a progeny with a numerically irregular relation of the sexes. On the contrary, the females which contain it, whether in a heterozygous or a homozygous state, nevertheless remain perfectly normal. This can be seen, for example, in the crosses of type III for the heterozygous females, and in the cross No. 1644 (and the like) for homozygous ones. In this way, the hereditary factor which causes the appearance of "female" cultures is manifested exclusively in the males; in other terms, it is not only sex-linked, but likewise sex-limited.

In order to penetrate somewhat deeper into the mechanism of its action, all the known cases of sex-ratio abnormalities are to be kept in view. The diversity of these deviations can be reduced to a comparatively small number of causes, which may be classified as follows:

I. Causes that influence the normal mechanism of distribution:

1. Parthenogenesis.
2. Unequal participation of different kinds of gametes in fertilization.

II. Causes exercising no influence on the normal mechanism of distribution:

1. Destruction of the zygotes of one sex through the injury of the gametes, which participate in fertilization.
2. Destruction of the zygotes of one sex, owing to their containing lethal factors.
3. Different resistance of the sexes to external influences.
4. A complete transformation of sex, as the extreme degree of intersexuality.

In order to decide to what group the phenomenon observed in *D. obscura* belongs, all the possibilities ought to be examined.

It is easy to establish at once, that the causes, enumerated for the first and the last categories (parthenogenesis and complete sex transformation) are totally inadequate to explain this abnormality. Parthenogenesis is entirely impossible, there being here a transmission of the X-chromosome from the father to its offspring (for instance, in the crossings of type II). The supposition that the observed abnormality is caused by a complete transformation of sex must also be rejected. Not once did I find in my cultures any individual bearing intersexual characters, although all the flies underwent a careful examination; neither did the anatomical dissection of numerous flies from the "female" cultures present any abnormalities. Further, a male of Drosophila, transformed into a female, must give two kinds of gametes—those with a Y and those with an X-chromosome. The combination with similar gametes of a normal male would present four types of zygotes: XX, 2XY and YY (the last perishes). Consequently, in the progeny of such transformed males a sex-ratio of 1 female to 2 males ought to be found—a thing that was never observed during the present work. Lastly, in some cases (for instance in the crosses of type II) the missing males are such as ought to be perfectly normal in regard to their genotypical structure; therefore, their transformation is of course highly improbable.

Passing on to other possibilities, it is necessary first of all to answer the question: Does one of the sexes perish in the process of development, or are both kinds of zygotes produced in different quantites from the very beginning? Happily, in Drosophila the problem can be solved. As is shown by the genetic and physiological experiments of other investigators, the female of Drosophila lays all the fertilized eggs, whether they

be capable of development or not. By counting the number of eggs laid and then that of the flies developed out of them, one can determine the percent of the embryos that have perished and even the stage when their death took place. Such researches have been more than once carried out with *D. melanogaster* and the methods have been sufficiently well elaborated.

Table 10 gives results of analogous experiments undertaken to answer

TABLE 10

SERIES	NUMBER	EGGS	LARVAE	FLIES	EGGS DIED (percent)	LARVAE AND PUPAE DIED (percent)	PERCENT ♂♂
Normal line	1780	108	106	74	1.9	30.2	51.4
	1783	84	80	58	4.8	27.5	46.6
	1785	127	127	89	0.0	29.9	44.9
	1936	105	101	69	3.8	31.7	47.8
	Total	424	414	290	2.4	30.0	47.6
Line 3	1940	125	123	85	1.6	30.9	5.9
	1943	135	130	81	3.7	37.7	2.5
	1944	85	85	55	0.0	35.3	3.6
	1951	98	98	77	0.0	21.4	5.9
	1959	67	64	49	4.5	23.4	6.1
	Total	510	500	347	2.0	30.1	4.6

this question in *D. obscura*. It was necessary firstly to determine the percent of perishing embryos in the cultures of the pure normal line; the number obtained were to serve as a control. The results showed that nearly all the eggs began to develop, and only 2 percent of them perished. On the contrary, the percent of larvae that succumbed was rather large; this may be attributed to the unsatisfactory nature of the diet. The experiments were carried on in a thermostat with a constant temperature of 25°C. The methods used were the same as described in the recent work of J. CHI LI (1927). A second series of experiments, carried out under the same conditions, was organized with flies homozygous for the gene which causes the appearance of "female" cultures. The results obtained here turned out to be extremely like the data given by the control; the percent of eggs and larvae that perished was almost exactly the same as in the normal line.

Thus, direct experiments, made in order to solve the question whether there exists in the "female" cultures of *D. obscura* a differential removal of one of the sexes in the course of development, give a clearly negative answer. Therefore it must be concluded that there is here a real alteration in the mechanism regulating the normal sex-destribution, that is, an alteration of the mechanism of sex-determination.

CONCLUSION

Analysis of the irregular sex-distribution in *D. obscura* shows that this phenomenon is due to the presence of a sex-linked gene. The action of this gene is analogous to that of a gametic lethal; the greater part of the spermatozoa determining the development of males do not participate in fertilization, consequently they are lost for the offspring. To say with greater precision on the strength of the results obtained, what constitutes the action of this gene, seems at present impossible, but nevertheless some suppositions on the subject can be made. Only two possibilities have to be reckoned with here: either the "female" progeny is determined by an unequal formation of spermatozoa with the X and Y-chromosomes during spermatogenesis; or else, even if the quantity of both kinds of spermatozoa formed be equal, the preponderance of females in the offspring can be explained by the inability of the sperms with the Y-chromosomes to compete with the sperms carrying the X. The last supposition seems more probable, as in this way the appearance of a certain number of males in the "female" cultures is easier to explain, but it is as yet impossible to decide finally to which of the two described types the observed phenomenon belongs. Anyhow, it can be considered as certain that the spermatozoa with the Y-chromosome participate in the fertilization process in but an insignificant number, while the spermatozoa carrying the X-chromosome (and therefore the gene that calls forth the abnormality) fecundate unhindered the great majority of eggs.

It is worth while to note that this gene was first obtained from nature. Out of 19 wild females which were caught two were heterozygous for it, thus giving us the right to suppose that it infects in a comparatively high degree the local *D. obscura* population. The gene is sex-linked, therefore it ought to respond to the influence of natural selection with particular directness, as no inbreeding is required for its manifestation. Hence the conclusion can be drawn that since it exists in the natural population it is probably useful, or at least harmless for the evolution of the given species. If this supposition be correct, the extension of this gene among the wild population must be expected even without the action of positive

selection. Usually a sex-linked gene is transmitted by the father to a half of the descendants only, whereas here all or nearly all the flies receive it with the X-chromosome of the father; this favors its extension.

It is interesting to note that an apparently quite similar sex-ratio abnormality was found by STURTEVANT in *Drosophila affinis* (MORGAN, BRIDGES and STURTEVANT, 1925). He gives the following description of this case: "The data obtained indicate that a great deficiency of sons was obtained from certain males, regardless of the source of the females to which such males were mated. The few males obtained from such matings were, usually at least, normal in behavior; but some of the sons of the females from such anomalous cultures again gave very few sons." Unfortunately this interesting stock was lost before a more detailed analysis of it could be undertaken, so that it is impossible to determine whether the causes of both the abnormalities are the same. However, it seems very probable that the mechanism of both cases must be identical at least in regard to its essential features.

SUMMARY

1. The sex-ratio in the normal lines of *Drosophila obscura* is very near to the theoretical 1:1.

2. Out of 19 females caught in nature, two were heterozygous for a gene which causes strong deviations in the normal sex distribution.

3. The researches made have shown that this gene is localized in the X-chromosome and is transmitted like an ordinary sex-linked gene.

4. This gene is absolutely sex-limited, as it is not manifested either in heterozygous or homozygous females.

5. The males bearing this gene give in their progeny about 96 percent of females and only about 4 percent of males.

6. This gene has no influence on the development of the zygotes already formed, but acts directly upon the mechanism of sex-distribution. It provokes a sharp preponderance of females by almost totally removing the spermatozoa with the Y-chromosome from the fertilization process, acting thus like a gametic lethal (in the genetic sense of this term).

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AN EIGHT-FACTOR CROSS IN THE GUINEA PIG*[1]

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THE STOCKS

An eight-factor cross in the guinea pig

The purpose of the present paper is to present data on the assortment, relative to each other and to sex, of eight pairs of Mendelian factors of the guinea pig. A stock, recessive in six color factors and in two factors which affect hair direction, was built up from various sources. This stock consisted of smooth furred, pink eyed, pale brown-yellow-white tricolors. A stock of self colored golden agoutis, smooth furred except for reversal of hair direction on the hind toes, was built up to supply the 8 dominant allelomorphs. The following are the series of factors involved.

S, s	s	piebald (white areas) (incomplete dominance
E, e^p, (e) e^p		tortoise shell (red areas), e self red
A, a	a	black in place of agouti
C, c^k, (c^d, c^r, c^a)	c^k	slight dilution of black, marked dilution of red
	c^d	marked dilution of both black and red
	c^r	slight dilution of black, white in place of red, red eyes
	c^a	white in place of both black and red, pink eyes (albino)

* The GALTON AND MENDEL MEMORIAL FUND pays part of the cost of the accompanying tables.

[1] A large portion of the data for this paper was obtained in experiments carried on by the author in the Bureau of Animal Industry, UNITED STATES DEPARTMENT OF AGRICULTURE.

B, *b*	*b*	brown in place of black, brown eyes, red unaffected
P, *p*	*p*	pale sepia in place of black, pink eyes, red unaffected
R, *r*	*R*	rough fur in place of smooth fur, at least on hind toes
M, *m*	*m*	extension of roughness to back and head (incomplete dominance of *M*)

DIFFICULTIES IN CLASSIFICATION

As might be expected, there were certain difficulties of classification among the 192 varieties expected in the backcross generation (*M*, *m* produce no visible effect except in the presence of *R*).

The piebald pattern (due primarily to *ss*) is subject to the action of modifiers which may cause the average percentage of white to vary from at least 10 to 90 percent. The average in the multiple recessive stock was 59 percent for males and 67 percent for females. There is also much variation which is not genetic and yet not due to environmental factors common to litter mates. Genetic piebalds (*ss*) occasionally show no white. It is not unlikely that homozygous self (*SS*) could be produced with a little white by selection for the appropriate minor factors. These cases were certainly so rare in the stocks used that there would have been no serious difficulty in classification into self and piebald were it not that dominance is incomplete. The heterozygotes are often completely self colored but rather more frequently they show a little white on nose or feet. Occasionally they range as high as 25 percent white in the coat, at least in females, with no more residual heredity for white than expected from the stocks used here. The distribution of percentages of white in the multiple recessive stock, in the F_1 matings of 3 homozygous dominant animals and in the backcross generation, are given in table 1. There is clearly some overlapping of the ranges of *Ss* and *ss* but separation of the males into "near self" and "piebald" at 7.5 percent white and of the females at 22.5 percent gives approximately equal numbers of the two classes and can involve no error that would seriously affect the recombination percentages.

The factor e^p (tortoise shell) was given preference over *e* (self red) in making up the multiple recessive stock, in order to avoid interference with the agouti series. Factor *e* was, however, carried in one of the foundation stocks, that used for introduction of *b*, and segregated out in a few backcross matings ($Ee \times e^pe$), in place of e^p. The distinction between the dominants (Ee^p) and the recessives (e^pe^p) from the typical backcross matings was ordinarily easy to make. There are rare cases (as noted by IBSEN 1919) in which animals which are undoubtedly e^pe^p show no red or yellow spotting in the coat, and more frequent cases in which there are

TABLE 1

The amount of white in the coat in (A) the multiple recessive stock (ss), (B) among F_1's from cross of multiple recessives with 3 multiple dominants proved to be homozygous (SS) by occurrence of no piebalds among 16, 24, and 26 young respectively, and (C) in the backcross generation excluding red-eyed dilutes.

MATING	SEX	AMOUNT OF WHITE IN COAT (parts in 20)																					
		0	x	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Multiple recessive stock (A) (*ss*×*ss*) 1924, 1925	♂	2	5	6	6	10	9	6	6	3	10	10	8	11	19	12	14	15	10	16	10	18	17	223
	♀	2	4	1	4	6	5	5	6	6	4	7	9	15	13	16	20	14	12	16	21	25	20	231
3 F_1 Matings (*SS*×*ss*) (B)	♂	16	15	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	31
	♀	14	19	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	35
Backcross (*Ss*×*ss*) (C) (*c*^{r}*c*^{r} omitted)	♂	33	70	9	8	8	7	11	6	5	2	5	3	6	4	7	5	2	5	6	3	4	2	211
	♀	10	65	5	8	7	9	12	10	4	12	3	9	3	5	16	4	7	10	8	6	11	2	226

only a few hairs of these colors. The close approach to equality of recorded Ee^p and e^pe^p among dark-eyed young (115:113) indicates that there was little or no error among these but the deficiency of tortoise shells among the pink eyed ($134Ee^p$:$98e^pe^p$), although not certainly significant, may indicate some tendency to overlook yellow hairs in these animals in which black and brown are reduced to colors which do not contrast sharply with yellow. The crossover figures involving E are accordingly reported separately for dark-eyed and pink-eyed young. Another possible source of error in the separation of Ee^p from e^pe^p and also of Ss from ss came in the few cases in which factor c^r segregated out as the representative of the albino series. This factor reduces the red spotting of tortoise shells to white. Fortunately the pattern is typically so different from piebald that classification is usually possible. Among the 21 animals of formula c^rc^r, 2 were pink-eyed whites, resembling albinos, but undoubtedly of formula eec^rc^rpp. These, of course, could not be classified with respect to piebald. Five had no white spotting of any sort and could thus only be called $SsEe^p$, 3 had a trace of white only in regions expected in heterozygous piebalds and were also called $SsEe^p$, 4 had white brindling characteristics of tortoise shells, with little or no piebald white, and were called Sse^pe^p, 4 had a large amount of white in typical piebald patterns only and were called $ssEe^p$, while 3 showed both typical piebald white and white brindling and were called sse^pe^p.

There was never any doubt about the classification into agouti (Aa) and non-agouti (aa), except in 12 animals which showed no dark pigment in the coat (ee, or e^pe^p with so much white that there happened to be no dark pigmented spot).

In the albino series, factor c^k was given preference over c^r and c^a as the recessive representative. Albinism (c^a) would have interfered with the expression of all of the other color factors and c^r raises the difficulty in the separation of piebald and tortoise shell noted above. Factor c^d, which dilutes black more obviously than does c^k, would have been preferable, but these factors were not distinguished until the somewhat long drawn out process of putting the recessive stock together had been nearly completed. It turned out then that c^d had been wholly lost from this stock which thus came to be largely c^kc^k but carried c^r sufficiently frequently to enable c^rc^r to appear in the back cross generation in the 21 animals referred to above (from matings $Cc^r \times c^kc^r$). There was no difficulty in distinguishing Cc^k from c^kc^k in cases in which there was red (Cc^k) or yellow (c^kc^k) in the fur, whether due to the agouti pattern (Aa) or to tortoise shell (e^pe^p). In animals of constitution Ee^paa, the grade of black or brown averages

somewhat lighter with $c^k c^k$ than with Cc^k but as previously shown (WRIGHT 1925, 1927) there is so much overlapping that classification is unsatisfactory. It has seemed best to omit all animals of this constitution in considering the relations of the C series.

There was no difficulty in distinguishing *Bb* and *bb* in animals which were not pink-eyed as both eye color and the difference between black and brown in the dark parts of the fur provide reliable criteria. Eye color fails in the pink-eyed varieties and the difference between pale sepia and pale brown is not always clear cut. It has seemed best to present the data for dark eyed (*Pp*) and pink eyed (*pp*) separately, although it is believed that there was no substantial amount of error in the latter.

There was never any difficulty in distinguishing *Pp* with black, brown or red eyes, from *pp* with pink eyes, albinism having been entirely absent from the recessive stock. A brown-pale brown-red-white quadricolor, mosaic in *P* and *p*, appeared in the backcross generation (WRIGHT and EATON 1926). This animal transmitted *P* in a mating with a pink-eyed animal and is here classified as *Pp*.

There was also no difficulty in distinguishing rough furred (*Rr*) from smooth (*rr*) (except in two animals of which only the heads were not eaten when found).

The rough furred fell easily into two approximately equal groups, full roughs, *Rrmm*, and partial roughs *RrMm*. The former have at least two pairs of dorsal rosettes, and rosettes about and between the eyes, while in the latter there is typically only one dorsal pair or even merely a dorsal crest and no head rosettes. A few doubtful, intermediate cases were assigned by drawing a somewhat arbitrary line. This factor *M* was derived from a different source from the modifier of full rough described in an earlier paper (symbol *S*, WRIGHT 1916). Nevertheless its behavior as followed in the production of the multiple dominant stock was exactly the same. Inasmuch as a similar modifier has been found homozygous in 3 wild species of cavy as well as in various domestic stocks of guinea pig there need be little hesitation in considering it as always the same. Smooths of formula *rrMM* are to be considered as "type" from which the full rough *RRmm* differs by 2 factors. In the production of the multiple recessive stock, all of the final stock (smooth) was derived by segregation from full rough parents (*Rrmm*×*Rrmm*) in order to insure the absence of factor *M*.

ABSENCE OF SEX LINKAGE

The first crosses between the multiple dominant and recessive stocks were made before the former had been rendered completely homozygous.

There was in consequence segregation of one or more factors in most cases. In a typical case of a mating between a male dominant and a female recessive the young consisted of 11 partial rough golden agouti (4 ♂, 7 ♀) and 9 partial rough cream agouti (5 ♂, 4 ♀), indicating a mating of type $SSEEAACc^aBBPPRRMM \times sse^pe^paac^kc^kbbpprrmm$. The production of males dominant in all 8 factors in this and other similar matings proves that all 8 of the factors are transmissible from father to son and nence not sex linked, if the male is heterozygous for sex as in cats and man and as indicated by cytologic evidence for a number of other mammals. The cytologic situation is not certain in the case of the guinea pig. An X-Y pair is reported in the primary spermatocyte by STEVENS (1911) and by HARMON and ROOT (1926) but had not been identified in work reported by PAINTER (1926). However, it is clear from reciprocal crosses (female dominant by male recessive) that none of the 8 factors show sex linkage of the type in which the female is heterozygous for sex. A typical mating of this sort gave 8 partial rough agoutis (2 ♂, 6 ♀) and 4 partial rough agouti tortoise shells (all males) indicating segregation of E and e^p in the mother. Transmission of all 8 factors from mother to daughter was found in many other cases besides the 6 from this cross.

THE MONOHYBRID RATIOS

Fourteen F_1 males and 17 F_1 females, all partial rough agoutis with a little white in the fur in most cases, were tested by backcrossing to the recessive stock. The results for each pair of factors separately are given, by matings, in table 2. The males A, B and C and females a to i came from the cross of male dominant with female recessive, while the others came from the reciprocal cross.

There are two cases (matings F and T) in which it is clear from the results that the F_1 parent was not a heterozygous dominant with respect to piebald, but a recessive (ss). One of these (T) had 15 percent white in his coat and came from an F_1 mating which was proved to be $Ss \times ss$ by the production of well defined piebalds. The amount of white made it more probable than not that he would turn out to be a recessive in this respect, although another male (H) with 15 percent (as well as the male of G with 10 percent) turned out to be heterozygous. The rest of the males had only 5 percent or less white in the coat. The breeding record of mating F makes it certain nevertheless that this male (no white in coat!) also was genetically a piebald. The progeny of these matings are used in studying the recombination percentages except those involving S.

TABLE 2

Segregations with respect to each pair of allelomorphs separately from each F_1 male and F_1 female (some mated 2 or 3 times). Male of YZ was mated with Rr females and hence with expectation of 3R-:1rr instead of 1:1. Males of F and T proved to be ss. Females of u, v, and w proved to be AABBPP.

	NUMBER	♂	♀	*S*	*s*	*E*	$e^p e$	*A*	*a*	*C*	c^{kr}	*B*	*b*	*P*	*p*	*R*	*r*	*M*	*m*
A, G	30	10	20	14	16	18	12	13	17	4	14	20	10	9	21	12	18	5	7
B	9	6	3	6	3	3	6	5	4	3	4	4	5	4	5	3	6	0	3
F	13	9	4	(0	13)	6	7	7	5	7	4	7	6	6	7	8	5	3	5
H	12	7	5	[4	6	3	9	2	4	6	4	5	4	6	6	6	6	1	5
I	28	13	14	16	11	14	13	11	17	11	9	17	11	14	14	14	14	7	7
J, Q, R	40	23	17	24	16	21	19	14	25	14	10	21	18	23	17	21	19	7	14
K	14	5	9	8	6	7	7	6	8	3	7	8	6	4	10	7	7	5	2
L, M	29	16	13	14	15	18	11	20	9	17	7	15	14	20	9	16	13	5	11
N	3	0	3	2	1	2	1	2	1	2	0	1	2	2	1	1	2	0	1
O	7	2	5	1	6	2	5	3	3	2	4	3	3	4	3	6	1	5	1
S, V	34	14	20	18	15	21	12	12	22	12	9	19	14	20	14	12	22	9	3
T	14	6	8	(0	14)	7	7	7	6	6	7	7	6	4	10	11	3	8	3
W, X	17	4	13	9	8	10	7	6	11	4	5	7	10	8	9	9	8	5	4
Y, Z	19	9	10	10	9	7	12	10	8	8	8	9	9	8	11	(12	7)	6	6
Total (1:1)	269	124	144	126	112	139	128	118	140	99	92	143	118	132	137	126	124	66	72
Dev.±SE		10.0±8.2		7.0±7.7		5.5±8.2		11.0±8.0		3.5±6.9		12.5±8.1		2.5±8.2		1.0±7.9		3.0±5.9	
Prob.		.22		.36		.50		.17		.61		.12		.76		.90		.61	
(0:1) & (3:1) unclassified		1		(0 4	27)	2		11		78		8				(12	7)		

a	34	17	17	18	16	16	18	17	17	17	11	18	16	18	16	17	17	8 9
b	6	4	2	2	4	3	3	3	3	2	2	1	5	5	1	4	2	2 2
c	24	16	8	11	13	16	8	16	8	10	9	13	11	9	15	12	12	8 4
d	2	1	1	1	1	1	1	2	0	0	2	1	1	2	0	1	1	1 0
e	3	1	2	0	3	1	2	3	0	2	1	1	2	0	3	0	3	0 0
f	3	2	1	2	1	3	0	2	1	1	1	1	2	2	1	2	1	1 1
h	4	1	3	0	4	2	2	2	2	0	3	2	2	2	2	3	1	1 2
i	7	6	1	3	4	6	1	2	5	2	1	2	5	3	4	4	3	3 1
j	5	3	2	3	2	1	4	3	2	1	3	2	3	3	2	3	2	2 1
k	17	8	8	8	9	13	4	10	7	6	6	13	4	10	7	8	9	3 5
l, r	15	5	10	8	7	10	5	9	6	6	4	7	8	4	11	7	8	6 1
m	16	7	8	6	9	8	8	10	6	10	2	7	9	8	8	4	11	4 0
n, s	26	11	14	15	11	14	11	16	9	11	9	13	12	16	10	18	7	6 12
o	21	8	13	10	11	9	12	7	14	5	9	8	13	9	12	8	13	1 7
p	3	3	0	1	2	2	1	1	2	0	2	2	1	2	1	3	0	1 2
t	8	2	6	5	3	5	3	3	5	3	2	2	6	3	5	4	4	2 2
u, v, w	27	14	13	12	15	10	17	(26	0)	15	12	(27	0)	(27	0)	9	18	5 4
Total (1:1)	221	109	109	105	115	120	100	106	87	91	79	93	100	96	98	107	112	54 53
Dev. ± SE		0.0±7.4		5.0±7.4		10.0±7.4		9.5±6.9		6.0±6.5		3.5±6.9		1.0±7.0		2.5±7.4		0.5±5.2
Prob.		1.00		.50		.18		.17		.36		.61		.88		.74		.92
(1:0) unclassified		3		1		1		(26 2	0)	51		(27 1	0)	(27	0)	2		
(1:1) ♂ + ♀ tests	490	233	253	231	227	259	228	224	227	190	171	236	218	228	235	323	236	120 125
Dev. SE		10.0±11.0		2.0±10.7		15.5±11.0		1.5±10.6		9.5±9.5		9.0±10.7		3.5±10.8		1.5±10.8		2.5±7.8
Prob.		.36		.85		.16		.89		.32		.40		.75		.89		.75

TABLE 3

Progeny from backcross test of F_1 males. The matings are indicated by letters as in table 2 and the litter by a subscript. Matings F, T, Y and Z are put in parentheses. F and T come from F_1 males which proved to be genetically piebald (ss) and the male of Y and Z was mated with heterozygous rough females. The symbols have the following meanings: B—Black; pBr—pale Brown; W—White; (Br)—Brown eye; Br—Brown; R—Red; Ag—Agouti; (P)—Pink eye; pS—pale Sepia; V—Yellow (or cream); (B)—Black eye; Sm—Smooth; RfA—Full Rough; RfC—Partial Rough. Such a combination as BrYAg-Y(Br) means brown yellow agouti with yellow spotting and (brown eye).

	NEAR SELF				PIEBALD							
	Full Rough		Partial Rough		Smooth		Full Rough		Partial Rough		Smooth	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
BRAg (B)		L_5		V_3	I_7	$J_3K_5L_3L_{12}$	M_2	Q_3				
BYAg (B)		I_9*	R_3	I_{10}		I_2*	(F_5)			V_3	$L_8(Y_1)$	A_8V_3
BrRAg (Br)	J_1		L_{12}						(Z_2)	H_2L_4	V_4	
BrYAg (Br)		A_2K_4			L_{10}						L_6	
B (B)	V_9	A_{10}	J_3	R_7S_1* V_9	Q_2X_2		(F_2)	X_3	$A_{10}M_2$	A_9V_8	$(F_1)I_6J_4L_9$	I_1*Q_3V_8
Br (Br)			L_1	J_4	$B_1K_7L_2$ R_7V_2	N_1Q_3	J_3	I_4		O_2	R_8V_3	K_7W_2
BRAg-R (B)		A_4S_1		O_1		N_1	L_2	R_8			L_7	
BYAg-Y (B)		Z_4			B_3I_4	H_3I_3*		(Z_1)		$A_7(T_3)$	A_7 I_6	O_1
BrRAg-R (Br)	$B_3(Y_2)$					$L_{11}X_1$					R_3	
BrYAg-Y (Br)					G_1J_4	B_2R_4	R_2					L_1W_1
B-R (B)	I_3R_3		X_3	$L_9W_5(Y_2)$	Q_2			(F_2)		(Y_3)		
B-Y (B)						W_5			V_1	(T_2)		
Br-R (Br)	M_1	I_5			$I_6V_5V_6$	$V_7(Z_3)$	H_3	(F_3)	I_6	$(T_3)_4$		V_4V_8
Br-Y (Br)	J_3		V_5	J_3					O_2			$L_7(T_2)$

pSRAg (P) pSYAg (P) pBrRAg (P) pBrYAg (P)		L_3 R_4 (Y_1)		(Y_4)	A_1A_6	K_1 V_6 L_3	(T_4) W_2 L_6 (F_4)	L_7 I_1 N_1 $B_3(T_1)V_8$	(T_3) G_1 (T_4)	(T_2) O_1 (F_5)X_3	 (F_5) V_5	(T_4) I_9V_2 A_4I_{10}
pS (P) pBr (P)	H_4 H_2I_9 X_1	R_5 A_2	 $I_7(Z_1)$	A_3 W_4	$S_1S_1V_6(Z_2)$ $A_3A_7A_9I_4$	V_2V_5 K_2V_3	 $R_2(Y_1)$	$A_1A_5G_1$ L_5W_3	 J_2	(T_2) K_2X_2	R_3Q_1 B_3	Q_1 $A_3I_{10}W_4$
pSRAg-R (P) pSYAg-Y (P) pBrRAg-R (P) pBrYAg-Y (P)	M_1 J_3	 L_4	I_7	I_7	$A_6K_8(Z_4)$ R_8	K_6 $A_1(Y_2)$ W_2X_2	B_2L_6 (Y_4)		(F_1)R_1 (F_4)	V_4	(F_1) Q_3 V_7	A_5
pS-R (P) pS-Y (P) pBr-R (P) pBr-Y (P)	 Q_2	R_5	I_4	I_8	$B_1I_2V_8$	A_6R_1 B_3R_6	I_7	K_4R_7*	O_2 K_3 T_1 K_3	K_5 (Z_4)	 H_4 (F_3)(Y_1) $L_6(T_2)$	A_5I_1 A_9 A_4A_8

* White (due to c^rc^r) in place of yellow (due to c^kc^{kr})

Miscellaneous

♀	R-W	(B)	Sm	se-CBPr-	(H_3)	♀	R-W	(P)	Sm	Se-C-pr-	(Q_2)
♂	R-W	(B)	RfA	se-CBPRm	(H_4)	♀	Y-W	(P)	RfA	se-c^k-pRm	(Q_2)
♀	R-W	(Br)	RfA	se-CbPRm	(H_3)	♀	Y-W	(P)	Sm	se-c-^{k}p (r)	(Y_3)
♀	R-W	(P)	Sm	Se-C-pr-	(H_3)	—	pS-	(P)	RfC	--a-BpRM	(I_2)
♂	W	(P)	Sm	-e-c^r-pr-	(H_2)	♀	pSWAg	(P)	Sm	--Ac^r-pr-	(S_1)
♂	W	(P)	Sm	-e-c^r-pr-	(H_4)	♂	Y-W	(P)	RfA	(s) e^p-c^k-pRm	(T_3)
						♀	Y-W	(Br)	Sm	(s) e^p-c^kbPr-	(F_4)

TABLE 4

Progeny from backcross test of F_1 females. The tested females are indicated by letters as in table 2 and the litters by a subscript. The record of female u (including v and w) homozygous in A, B and P is given separately below. Color symbols as in table 3.

	NEAR SELF						PIEBALD					
	Full	Rough	Partial	Rough	Smooth		Full	Rough	Partial	Partial	Rough	Smooth
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
BRAg (B)			f_1			c_6	n_6		a_4			k_3
BYAg (B)	n_1	a_5		n_3	$k_6{*}o_7$	d_1			r_4			h_2t_3
BrRAg (Br)		o_5	C_5						a_6c_3	n_1		b_1k_6
BrYAg (Br)	p_1				f_1	a_9			m_4	a_{10}		k_3n_5
B (B)	k_5				k_2	l_1	s_2	s_3			c_4	n_2s_2
Br (Br)	a_8	0_1	i_4i_4		c_2m_4	o_4s_4			b_2			m_3o_8
			n_1									
BRAg-R (B)								c_5			$a_{10}t_2$	a_5m_2
BYAg-Y (B)			$b_1 j_3{*}$					c_4s_2	a_2			
BrRAg-R (Br)	b_2				m_1	m_6	a_9			j_3	m_{37}	
BrYAg-Y (Br)	n_2				c_2o_1	l_1n_6	o_5	$k_5{*}$	d_1		j_3	
B-R (B)	k_6		i_1		k_3	$a_{11}m_1r_1$						a_{11}
B-Y (B)	a_3						a_8	o_3	$p_1{*}$	c_4		
Br-R (Br)	n_5				a_3a_5			t_3				
Br-Y (Br)		a_1			a_2				h_1			b_2 o_7
pSRAg (P)	a_7	s_1	r_3	a_2	c_5	k_1m_1	k_2				$c_8i_1m_3$	c_2
pSYAg (P)	c_3		k_4		a_5					r_2	c_3	
pBrRAg (P)	n_2		r_1			a_3t_1		r_3		m_6		
pBrYAg (P)		n_3			c_6	c_1					e_1k_6	$a_1c_2i_3$

pS (P) pBr (P)	c_7j_2*		c_1 t_2*	a_7 $a_{11}o_2$ t_4	a_3l_1	a_3 t_4*	p_1 b_2i_2	h_2*k_3 f_1	c_4r_2	k_4* m_5	$a_1m_4o_6$ o_1i_1	o_3r_4 r_1
pSRAg-R (P) pSYAg-Y (P) pBrRAg-R (P) pBrYAg-Y (P)	n_5	n_3 o_4		n_6 r_3	m_6o_8	l_1		0_3 h_1	s_1 c_6	c_5 a_4	a_6	e_1 k_5r_1 a_7e_1
pS-R (P) pS-Y (P) pBr-R (P) pBr-Y (P)		a_{10} t_2*			c_7 n_3 o_6	o_7 j_1*		a_4 o_8	c_8	s_4 m_5	a_5o_8	o_2

* White (due to c^rc^r) in place of yellow (due to c^kc^{kr}).

BRAg (B) BYAg (B) BRAg-R (B) BYAg-Y (B)		u_8		u_4 u_3u_5	u_1u_4 u_1u_5 u_3u_3	u_5 u_9	u_7 u_5	u_7	u_2 u_8		u_2 u_{10} u_6u_7	u_6 $u_{10}u_{10}$ $u_2u_7u_9$

no BRAg spot present.

Miscellaneous

♂ R-W (Br) Sm se-CbPr- (s_2)
— pSRAg (P) -S-AC-p-- (s_3)
— pSRAg -R-W (P) - -e^pACB^p-- (m_2)
— B (B) RfC SEc-BPRM (k_5)

One F_1 female (mating r) produced no non-agoutis (*aa*) no browns (*aa*) and no pink-eyed (*pp*) among 27 young and was clearly *SsEe^{v}AACc^k BBPPRrMm*. There was no doubt about her identity because of a certain peculiarity in her rosette pattern recorded at birth and there was nothing in the records to indicate that her dam (a multiple dominant) had ever mated with any other male than her reputed sire (a multiple recessive). Whatever may be the explanation, it has seemed safe to include her progeny in the recombination data except where A, B and P are concerned.

One of the F_1 males was mated with two females which were full roughs (heterozygous) although recessive in the seven other respects (matings Y and Z). The progeny are used in estimating all crossover percentages except those involving *R*.

Examination of the totals in table 2 shows that the monohybrid ratios are all reasonably close to 1:1 although as noted above it is not unlikely that a few genetic tortoise shells are classed as self. The chance of obtaining a more divergent system of values for the 9 cases (including sex) is .85 by the χ^2 method (9 degrees of freedom). It must be remembered, however, that in the case of *S,s* and *M,m*, the overlapping classes were separated somewhat arbitrarily.

THE DIHYBRID RATIOS

The results of the backcross generation from the F_1 males are given in detail in table 3, the mating and litter of each animal being indicated by the entries. Table 4 deals similarly with the progeny of the F_1 females. All of the data are included either in the body of the table or below. The cases which cannot be used for every crossover test are indicated as described in the titles.

The combinations suitable for determining possible linkage relations between each pair of factors are abstracted in tables 5 and 6 together with the calculations of the crossover percentages and the chances of exceeding each by random sampling. Inspection of these reveals not one, either in the male or female tests, which can be said to differ significantly from 50 percent. The most probable cases of linkage among the male tests are those of *AM* and *BR* with indicated crossover percentages of 43.4 and 45.3 percent respectively. The chance of exceeding by random sampling are .08 and .14 in the two cases. The reality of these cases is not confirmed by the female test. The strongest indication among the latter is that of *C* and *B*, 45.8 percent with a probability of being exceeded by chance of about 1 in 3 and no confirmed by the close approach to 50 percent (49.2 percent) among the males. It will be seen that the deviations

TABLE 5

The combination of each two sets of allelomorphs in backcross tests of F_1 males. The two original combinations are indicated in the column headings by XY and xy (double dominant and double recessive respectively) while the cross combinations are indicated by Xy and xY following in the order of the factors in the first column. The backcross ratio (1:1:1:1) is disturbed in the case of EC and AC by omission of Ea in which the separation of C and c^k was not satisfactory and in the case of RM by omission of smooths in which M and m produce no visible effect at all. The last column gives the probability of obtaining at least as great a deviation from 50% in either direction by random sampling. The value of χ^2 from the numbers of crossovers and linkages is 20.0 indicating a probability of .86 of obtaining at least as great deviations by random sampling (28 degrees of freedom).

	XY	Xy	xY	xy	TOTAL NO.	CROSSOVERS		PROBABILITY
						No.	Percent	
SE	64	62	62	50	238	124	52.1	.52
SA	56	68	47	60	231	115	49.8	.95
SC	49	38	37	40	164	75	45.7	.27
SB	70	54	58	52	234	112	47.9	.51
SP	68	58	54	58	238	112	47.1	.36
SR	51	65	55	48	219	120	54.8	.16
SM	26	31	28	33	118	59	50.0	1.00
EA	62	77	55	62	256	132	51.6	.62
EC	32	30	67	61	190	97	51.1	.77
EB	73	66	69	52	260	135	51.9	.54
EP	66	73	66	62	267	139	52.1	.50
ER	70	62	55	61	248	117	47.2	.37
EM	35	40	30	32	137	70	51.1	.80
AC	57	61	37	25	180	98	54.4	.23
AB	68	49	73	67	257	122	47.5	.42
AP	59	59	69	71	258	128	49.6	.90
AR	54	54	68	64	240	122	50.8	.80
AM	25	36	41	32	134	77	57.5	.08
CB	55	42	48	38	183	90	49.2	.82
CP	53	46	42	50	191	88	46.1	.28
CR	52	39	37	47	175	76	43.4	.08
CM	26	32	20	21	99	52	52.5	.61
BP	72	71	60	58	261	131	50.2	.95
BR	74	60	50	59	243	110	45.3	.14
BM	40	39	26	31	136	65	47.8	.61
PR	62	62	64	62	250	126	50.4	.90
PM	35	33	31	39	138	64	46.4	.39
RM	60	66	..	..	126	66	52.4	.59

TABLE 6

The combination of each two sets of allelomorphs in the backcross tests of F_1 females. Headings as in table 5. The value of χ^2 from the numbers of crossovers and linkages is 13.2 indicating a probability of .992 of obtaining at least as great deviations by random sampling (28 degrees of freedom).

	XY	Xy	xY	xy	TOTAL NUMBER	CROSSOVERS		PROBABILITY
						Number	Percent	
SE	60	44	60	55	219	104	47.5	.46
SA	49	44	56	43	192	100	52.1	.56
SC	45	35	45	44	169	80	47.3	.49
SB	42	50	50	50	192	100	52.1	.56
SP	48	45	48	52	193	93	48.2	.61
SR	51	53	56	59	219	109	49.8	.94
SM	25	26	29	27	107	55	51.4	.77
EA	59	51	46	36	192	97	50.5	.89
EC	37	32	53	47	169	85	50.3	.94
EB	54	56	39	44	193	95	49.2	.83
EP	49	61	47	36	193	108	56.0	.10
ER	60	60	47	52	219	107	48.9	.74
EM	33	27	21	26	107	48	44.9	.29
AC	56	50	19	17	142	69	48.6	.74
AB	52	53	41	46	192	94	48.9	.77
AP	53	53	42	45	193	95	49.2	.83
AR	52	52	46	41	191	98	51.3	.72
AM	27	25	22	24	98	47	48.0	.69
CB	40	35	30	37	142	65	45.8	.31
CP	35	41	40	27	143	81	56.6	.11
CR	39	50	40	49	168	90	53.6	.35
CM	21	18	18	22	79	36	45.6	.43
BP	45	48	51	49	193	99	51.3	.72
BR	45	47	53	47	192	100	52.1	.56
BM	20	25	29	24	98	54	55.1	.31
PR	49	47	49	47	192	96	50.0	1.00
PM	24	25	25	24	98	50	51.0	.84
RM	54	53	..	..	107	53	49.5	.92

from 50 percent are even smaller on the whole than might be expected by chance.

As already noted, the classification of factors *E* and *B* was easy in dark-eyed animals but was more difficult and accompanied occasionally by a feeling of uncertainty in the pink-eyed ones. It seems desirable therefore to examine the crossover percentages of these groups separately. The

TABLE 7

The crossover percentages from tests of F_1 males in the cases involving E and B, divided according to the presence of Pp or pp. In the former case, separation of Ee^p from E^pe^p and Bb from bb was easy and wholly reliable. In the latter cases there was more difficulty and occasionally uncertainty.

	BLACK OR BROWN EYED YOUNG (*Pp*)				PINK EYED YOUNG (*pp*)			
	Total Number	Crossovers		Prob.	Total Number	Crossovers		Probability
		Number	Percent			Number	Percent	
SE	122	65	53.3	.47	116	59	50.9	.85
SB	122	62	50.8	.86	112	50	44.6	.25
EA	128	67	52.3	.60	128	65	50.8	.86
EC	95	52	54.7	.36	95	45	47.4	.61
EB	132	58	43.9	.16	128	77	60.2	.02
EP	132	66	50.0	1.00	135	73	54.1	.34
ER	124	62	50.0	1.00	124	55	44.4	.21
EM	68	30	44.1	.33	69	40	58.0	.19
AB	128	55	43.0	.11	129	67	51.9	.66
CB	95	50	52.6	.61	88	40	45.5	.39
BP	132	60	45.5	.30	129	71	55.0	.25
BR	124	55	44.4	.21	119	55	46.2	.41
BM	68	32	47.1	.63	68	33	48.5	.81

TABLE 8

The crossover percentages from tests of F_1 females in cases involving E and B, divided according to the presence of Pp or pp. In the former case separation of Ee^p from e^pe^p and Bb from bb was easy and wholly reliable. In the latter case there was more difficulty and occasionally uncertainty.

	BLACK OR BROWN EYED YOUNG (*Pp*)				PINK EYED YOUNG(*pp*)			
	Total Number	Crossovers		Probability	Total Number	Crossovers		Probability
		Number	Percent			Number	Percent	
SE	123	57	46.3	.42	96	47	49.0	.84
SB	96	51	53.1	.54	96	49	51.0	.84
EA	95	46	48.4	.76	97	51	52.6	.61
EC	102	50	49.0	.84	67	35	52.2	.71
EB	96	48	50.0	1.00	97	47	48.5	.76
EP	96	47	49.0	.84	97	61	62.9	.01
ER	123	59	48.0	.65	96	48	50.0	1.00
EM	58	24	41.4	.19	49	24	49.0	.89
AB	95	50	52.6	.61	97	44	45.4	.36
CB	75	37	49.3	.91	67	28	41.8	.18
BP	96	51	53.1	.54	97	48	49.5	.92
BR	96	46	47.9	.68	96	54	56.3	.22
BM	49	26	53.1	.67	49	28	57.1	.32

necessary data are given in tables 7 and 8. The crossover percentages are somewhat more variable, as expected with the smaller numbers, but none differ significantly from the expectation under random sampling either in the dark-eyed or pink-eyed, except perhaps the figure of 62.9 percent for E and P in pink-eyed young in the female test and that of 60.2 percent

TABLE 9

The crossover percentages from tests of F_1 males and F_1 females combined. The value of χ^2 from the numbers of crossovers and linkages is 12.25 indicating a probability of .995 of obtaining at least as great a series of deviations from equality by random sampling (28 degrees of freedom).

	TOTAL NUMBERS	CROSSOVERS		PROBABILITY
		Number	Percent	
SE	457	228	49.9	.96
SA	423	215	50.8	.73
SC	333	155	46.5	.21
SB	426	212	49.8	.92
SP	431	205	47.6	.31
SR	438	229	52.3	.34
SM	225	114	50.7	.84
EA	448	229	51.1	.64
EC	359	182	50.7	.79
EB	453	230	50.8	.74
EP	460	247	53.7	.11
ER	467	224	48.0	.38
EM	244	118	48.4	.61
AC	322	167	51.9	.50
AB	449	216	48.1	.42
AP	451	223	49.4	.81
AR	431	220	51.0	.66
AM	232	124	53.4	.29
CB	325	155	47.7	.41
CP	334	169	50.6	.83
CR	343	166	48.4	.55
CM	178	88	49.4	.88
BP	454	230	50.7	.78
BR	435	210	48.3	.47
BM	234	119	50.9	.79
PR	442	222	50.2	.92
PM	236	114	48.3	.60
RM	233	119	51.1	.74

for E and B in the pink-eyed young in the male test. The former figure may readily be explained by the failure to recognize a small yellow patch in a few pink-eyed tortoise shells.

As only slight differences have been found in the crossover percentages of males and females in mammals (rats, mice and rabbits) it is probably legitimate to combine the male and female tests. The results are given in table 9. Here again there is no case which differs significantly from 50 percent, the extremes being 46.5 percent and 53.7 percent. The probability of obtaining a system of at least as great deviations (χ^2 test) by random sampling is the embarrassingly high figure of .995. The grand average recombination percentage for the 28 comparisons is 49.9 percent. It appears that for the present these 8 factors and sex must be considered as isolated representatives of 9 different linkage systems. There remain, of course, the possibility that some factors may be found showing appreciable linkage with two of the above and thus binding them into the same system.

THE OCTOHYBRID RATIO

Before leaving the data there are two other aspects from which it will be desirable to examine the randomness of assortment. First consider the combinations with respect to all 8 factors instead of merely by 2's.

The data are condensed in table 10 by disregarding the sex of the F_1 parent and of the offspring. Matings F, T, Y, Z and v are omitted because of the imperfections discussed above and mating D because of the production of 6 self reds in which classification by the agouti series could not be made. The few other imperfect individual records are also omitted. The 398 young left include 143 of the 168 distinguishable varieties expected in the backcross generation. Among the 96 classes in which expectation is 1/256 (or 1.56 in 398) there is variation from 0 to 4 individuals. Among the 56 classes with expectation 2/256 (or 3.11 in 398) there is variation from 0 to 8. The 8 classes with expectation of 4/256 (6.22 in 398) vary from 4 to 11 individuals. As these variations may appear greater than probable, it is worth while testing them against the distribution expected under random sampling which is approximately that of the Poisson series for mean values of 1.56, 3.11 and 6.22 respectively. Table 11 shows the comparison between observation and expectation on this basis. The χ^2 test, combining adjacent groups so as to leave none smaller than 4, indicates a probability of getting at least as great a system of divergences by random sampling of .33. Clearly there are no important departures from a random distribution.

TABLE 10

Segregation in backcross generation, combining tests of F_1 males and F_1 females, ignoring sex and omitting records of two males (F, T) found to be ss, of one male (H) found to be Ee instead of Ee^p and producing 6 ee young unclassifiable with respect to A and a, and one female (of u, v, w) proved to be AABBPP, and one male (YZ) which was mated with females heterozygous for Rough (Rr). All of the young from the other matings are included except 7 which were unclassifiable in one or more respects.

CHARACTERS		GENES	1 NEAR SELF			PIEBALD		
			Rf AB	Rf CD	Smooth	Rf AB	Rf CD	Smooth
			SRm	SRM	Sr-	SRm	sRM	sr-
BRAg	(B)	*EACBP*	1	2	6	3	1	1
BYAg	(B)	*C^kB*	3	3	4	0	2	5
BrRAg	(Br)	*Cb*	2	2	0	0	4	3
BrYAg	(Br)	*c^kb*	3	0	3	0	2	3
B	(B)	*Ea−BP*	3	5	4	3	4	9
Br	(Br)	*−b*	2	5	11	2	2	6
BrRAg−R	(B)	*e^pACBP*	2	1	1	3	0	5
BYAg−Y	(B)	*c^kB*	1	2	3	2	2	3
BrRAg−R	(Br)	*Cb*	2	0	4	1	1	2
BrYAg−Y	(Br)	*c^kb*	1	0	8	3	1	3
B−R	(B)	*e^paCBP*	3	4	5	0	0	1
B−Y	(B)	*c^kB*	1	0	1	2	3	0
Br−R	(Br)	*Cb*	3	0	6	1	2	2
Br−Y	(Br)	*c^kb*	2	2	1	0	2	3
pSRAg	(P)	*EACBp*	3	2	4	2	0	4
pSYAg	(P)	*c^kB*	1	1	4	2	3	1
pBrRAg	(P)	*Cb*	2	1	3	3	2	2
pBrYAg	(P)	*c^kb*	1	0	2	2	0	8
pS	(P)	*Ea−Bp*	3	3	6	8	1	8
pBr	(P)	*−b*	3	6	9	4	6	7
pSRAg−R	(P)	*e^pACBp*	1	1	3	2	3	3
pSYAg−Y	(P)	*c^kB*	1	1	3	1	0	2
pBrRAg−R	(P)	*Cb*	2	2	1	0	2	3
pBrYAg−Y	(P)	*c^kb*	2	0	3	1	1	1
pS−R	(P)	*e^paCBp*	1	1	6	2	1	4
0S−Y	(P)	*c^kB*	1	1	3	2	2	2
pBr−R	(P)	*Cb*	1	0	2	0	1	0
pBr−Y	(P)	*c^kb*	1	0	1	1	3	3

COMPARISON OF LITTERMATES

It is next desirable to see whether the data show any tendency toward resemblance of littermates in factorial constitution. In particular it is

TABLE 11

Distribution of class frequencies in Table 10 under each mean Mendelian expectation (1/156, 2/256, or 4/256) compared with random distribution as calculated from Poisson series with means of 1.56, 3.11 and 6.22 respectively. χ^2 for 12 degrees of freedom is 11.44 indicating a probability of 0.33 of obtaining at least as great deviations from theory by random sampling.

NUMBER IN CLASS	CLASSES WITH MEAN MENDELIAN EXPECTATION OF								
	1/256 1.56 in 398			2/256 3.11 in 398			4/256 6.22 in 398		
	O	C	O−C	O	C	O−C	O	C	O−C
0	22	20.3	+1.7	3	2.9		0	0.0	
1	29	31.5	−2.5	10	8.9	+1.2	0	0.1	
2	29	24.5	+4.5	10	13.8	−3.8	0	0.3	
3	14	12.7	+1.3	20	14.3	+5.7	0	0.6	
4	2	4.9	−5.0	8	11.1	−3.1	1	1.0	
5	0	1.5		5	6.9	−1.9	0	1.2	
6	0	0.4		5	3.6	+1.9	2	1.3	−1.5
7	0	0.2		0	1.6		1	1.1	+1.5
8	0			3	0.6		1	0.9	
9	0			0	0.2		2	0.6	
10	0			0	0.1		0	0.4	
11	0			0			1	0.5	
Total	96	96.0		64	64.0		8	8.0	
Individuals	137			201			60		

important to determine whether the number of littermates which resemble each other in all factors, including sex, is so great as to indicate the frequent occurrence of uniovular twins. Table 12 shows the number of agreements between littermates classified as ♂♂, ♂♀ and ♀♀ with respect to the S, E, A, B, P and R series. The M series is omitted because the possibility of classification of two littermates in this respect requires that both be rough furred and the C series is omitted for similar reasons. Matings F, T, Y, Z, and U are, of course, omitted. The symmetry of the results about 3 agreements, 3 disagreements in each case indicate that agreement is largely by chance. This has been tested by combining all into one table treating sex as a 7th factor and comparing the distribution with the expansion of $(\frac{1}{2}A+\frac{1}{2}D)^7$ where A and D are agreements and disagreements respectively. The close approach of the observed data to the theoretical distribution is obvious and is confirmed by a probability from χ^2 of .90. There is thus no tendency for littermates to resemble each other more than animals picked at random.

TABLE 12

Distribution of agreements and differences between littermates in sex and the six series S, E, A, B, P and R. Comparison is made with expectation from random sampling based on the expansion of $(\frac{1}{2}A+\frac{1}{2}D)$.[7] *Matings F, T, Y, Z and u, v, w, are omitted because of inequality of chances of agreement and difference in one or more of the above respects. The probability of obtaining at least as great deviations from theory by random sampling is 0.90 from* $\chi^2=1.65$ *for 5 degrees of freedom.*

AGREE	DIFFER	♂♂	♂♀	♀♀	TOTAL	*Calculated*	DIFFERENCE
0	7	..	3	..	3	*3.1*	−0.1
1	6	2	17	0	19	*21.3*	−3.2
2	5	9	49	11	69	*64.0*	+5.0
3	4	25	50	25	100	*106.6*	−6.6
4	3	32	45	28	105	*106.6*	−1.6
5	2	25	16	25	66	*64.0*	+2.0
6	1	6	3	17	26	*21.3*	+4.7
7	0	2	..	0	2	*3.1*	−1.1
		101	183	106	390	*390.0*	0.0

There were, however, two cases in which there was agreement in sex and in the 6 factors. There was indeed an 8th agreement in each case. Two male littermates, both brown with a trace of white were both partial roughs and thus both ♂*SsEe*^{p}*aabbPpRrMm* with uncertainty only in the albino series. Two other male littermates were both smooth pink-eyed pale sepias, one with no white, the other with a trace. While differing distinctly in grade of sepia, both were so pale as to be probably c^kc^r (both ♂*SsEe*^{p}*aac*^{k}*c*^{r}*BBpprr*). These two cases may have been uniovular twins but as 3 cases of agreement in all of the respects dealt with in table 12 are expected by chance, little confidence can be placed in this. It is certain that such twins are so rare as to be of no importance in disturbing the randomness of Mendelian results.

DISCUSSION

The evidence for random assortment of *S*, *E*, *A*, *C*, *B*, *P*, *R*, *M* and sex, presented here is in harmony with the rather scanty data hitherto published. CASTLE (1916) found a close approach to random assortment in 108 F_2 animals from a cross of golden agouti (wild Cavia cutleri) (*EEAACCBB*) and albino guinea pig, *eeaac*^{a}*c*^{a}*bb*. There was also random assortment in 116 F_2's from a cross of *Cavia Cutleri* with brown-eyed creams, *eeaac*^{d}*c*^{d}*bb* as far as *E*, *A*, and *B* were concerned, classification by intensity (*C*, c^d) not having been attempted. The present writer (1916) found 32/75=43 percent recombination between *R* and *M*, 34/53=64

percent between R and A and $35/64 = 55$ percent between A and M, and found recombination to occur between R and E, B, C and P and between M and B, C, P and in a later paper (1923) noted the completely random assortment S and E in 11 successive backcrosses to an inbred tricolor stock. IBSEN (1923) reported the following ratios of non-crossovers to crossovers:

	N^*	X^*		N^*	X^*		N^*	X^*
EA	5	10	AC	7	4	CP	65	67
EC	43	55	AB	9	4	CR	22	27
EB	19	9	AP	7	9	BP	10	18
EP	82	73	AR	9	5	BR	10	11
ER	29	31	CB	11	6	PR	47	28

*n = noncrossover. x = crossover.

IBSEN and GREGORY (1924) report independent assortment of P and a factor Sm (salmon eye) not belonging to any of the series discussed here and the present writer (1927) has found no linkage between another color factor, F and factors C and P among 242 F_2's, which data incidentally gave further evidence of the complete independence of factors C and P of each other.

The most interesting cases are perhaps this case of C and P and that of C and B. Factors with effects similar to those of C and P in the guinea pig have been found to be linked in both the mouse and rat with crossover percentages between 10 and 20 percent in both cases (CASTLE 1919). The albino series (C) is linked with the brown series (B) in the rabbit (41 percent crossover) (CASTLE 1924). The apparent absence of linkage in these cases in the guinea pig does not necessarily prove that the factors are not homologous in the different rodents as there may well have been radical chromosome reorganizations since their remote origin from common ancestry, a phenomenon indicated by probable differences in chromosome number.

The chromosome number in guinea pigs, however, seems to be far from certain. Estimates published in the past 20 years range from 8 for the haploid number (LAMS 1913) to 30 (PAINTER 1926). STEVENS (1911) found 28 and HARMON and ROOT (1926) found 19. The independence of the first 9 factors tested in the guinea pig (including sex) is difficult to reconcile, with a haploid count of 8 but is not at all surprising if one of the larger numbers proves correct.

SUMMARY

Production of two stocks of guinea pigs carrying 8 dominant and 8 recessive factors, respectively, made possible a simultaneous test for possible linkage relations between each two of the factor series designated by S, E, A, C, B, P, R, and M.

A backcross generation of 269 individuals from the (largely) octohybrid males showed completely random assortment and similarly with a backcross generation of 221 from (largely) octohybrid females. None of the factors showed sex linkage either of the XY type or the ZW type.

143 of the 168 visibly distinct combinations expected in the backcross generation were obtained among 398 young from completely octohybrid backcrosses. It is shown that the distribution of the combinations, considering all factors at once, is random.

There is no indication of a tendency for littermates to resemble each other more than expected by chance. Two possible cases of uniovular twins were found where three cases of resemblance in all non-interfering respects was expected by chance.

The independence of the first 9 factors tested is not surprising if the large number of chromosomes reported for the guinea pig recently is correct.

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A SEX DIFFERENCE IN CHROMOSOME LENGTHS IN THE MAMMALIA[1]

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During the last two years we have had occasion to accurately draw and enlarge human chromosomes. Both male and female, embryonic and adult, tissues have been studied. In surveying the first or longest chromosomes of the series we were early struck by the greater length of this chromosome in the male. Five independent series of measurements have furnished invariable confirmation of the earlier observations.

There are many pitfalls for the investigator who aims at accuracy and comparable results with the mensuration of chromosomes. Prophase chromosomes are invariably slightly longer than those assembled at the equatorial plate after dissolution of the nucleus; varying methods of fixation and dehydration shrink tissues unequally; and finally there are differences in the length and size of chromosomes in various cells especially in embryonic cells as compared with adult ones. It is rather interesting that different types of cells vary somewhat in chromosome length as they undoubtedly do in the size of the cell itself. A striking instance of this is found in the lutein cells in the corpora lutea of the ovary at the time of pregnancy. The cells are greatly enlarged and the chromosomes have a corresponding increase in size, the length and diameter being approximately two or more times those of the chromosomes of the surrounding cells. For all of these reasons we have sought to compare chromosome lengths only in the same phase of mitosis (late prophase) from the same type of tissue (mesenchyme) in embryos of approximately the same age, fixed and dehydrated by identical processes. Many human embryos of both sexes were explored for our work, but an immediate fixation (at the operating table) with resulting clarity of the chromosomes was secured in only two embryos of approximately the same age; a male of G. L. 19.5 to 20.5 mm and a female of G. L. 25 mm.

In both cases we selected ten nuclei of the mesenchyme situated near the ectoderm, displaying clearly all of the forty-eight chromosomes, which were then individually drawn at 3600 diameters. The original

[1] This work has been greatly aided by a grant from the Committee for Research in Problems of Sex of the NATIONAL RESEARCH COUNCIL. We would also thank Dr. SYLVIA L. PARKER for advice in some aspects of the statistical arrangement of the data.

drawings were enlarged twice photographically. From these records measurements were made of the chromosome lengths, which were determined by measurement of the median axis in every case.

There is a clearly marked sex difference in the length of the first (or longest) pair of chromosomes. The mean length of the two longest chromosomes from the ten male nuclei exceeded by 8.22 mm the mean length of the longest female pair. The probable error of this difference is + .74 mm. The difference is thus over ten times the probable error of the difference. It indicates that the longest autosomes in this male embryo

TABLE 1

Individual measurements (×7200) of extreme autosomes (in mm) of ten nuclei from human embryos of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	37.6	37.5	23.7	23.5	7.5	8.3	9.3	9.7
2.	38.9	33.0	35.6	31.0	8.1	9.0	10.3	11.3
3.	39.6	33.5	28.0	25.2	6.5	8.0	10.0	10.6
4.	38.2	36.1	31.1	28.0	7.3	9.0	8.7	9.1
5.	38.5	36.1	34.2	30.2	9.1	9.5	8.7	9.7
6.	38.0	34.7	39.3	27.2	7.8	8.5	9.5	9.5
7.	41.0	40.7	28.7	28.5	10.0	10.0	10.3	10.7
8.	40.4	37.5	36.5	31.0	8.0	9.8	10.0	10.1
9.	45.6	37.7	30.7	29.0	8.1	8.7	9.0	9.0
10.	39.5	38.5	30.0	26.7	8.7	9.0	10.2	11.2
Average	39.7	36.5	31.8	28.0	8.1	9.0	9.6	10.1

TABLE 2

Human embryos (10 nuclei of each sex; measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	38.13 ± .41	2.73 ± .29
Longest pair ♀	29.91 ± .62	4.08 ± .44
Difference ♂ − ♀	8.22 ± .74	−1.35 ± .51
Average of all 46 autosomes ♂	18.79 ± .24	7.46 ± .17
Average of all 46 autosomes ♀	17.23 ± .16	5.17 ± .11
Difference ♂ − ♀	1.56 ± .30	2.29 ± .21
Shortest pair ♂	8.55 ± .13	.90 ± .10
Shortest pair ♀	9.86 ± .11	.72 ± .08
Difference ♂ − ♀	−1.31 ± .17	.18 ± .12

were significantly longer than the longest autosomes in this female embryo. The data are given in table 9, and the constants computed from them, in table 2.

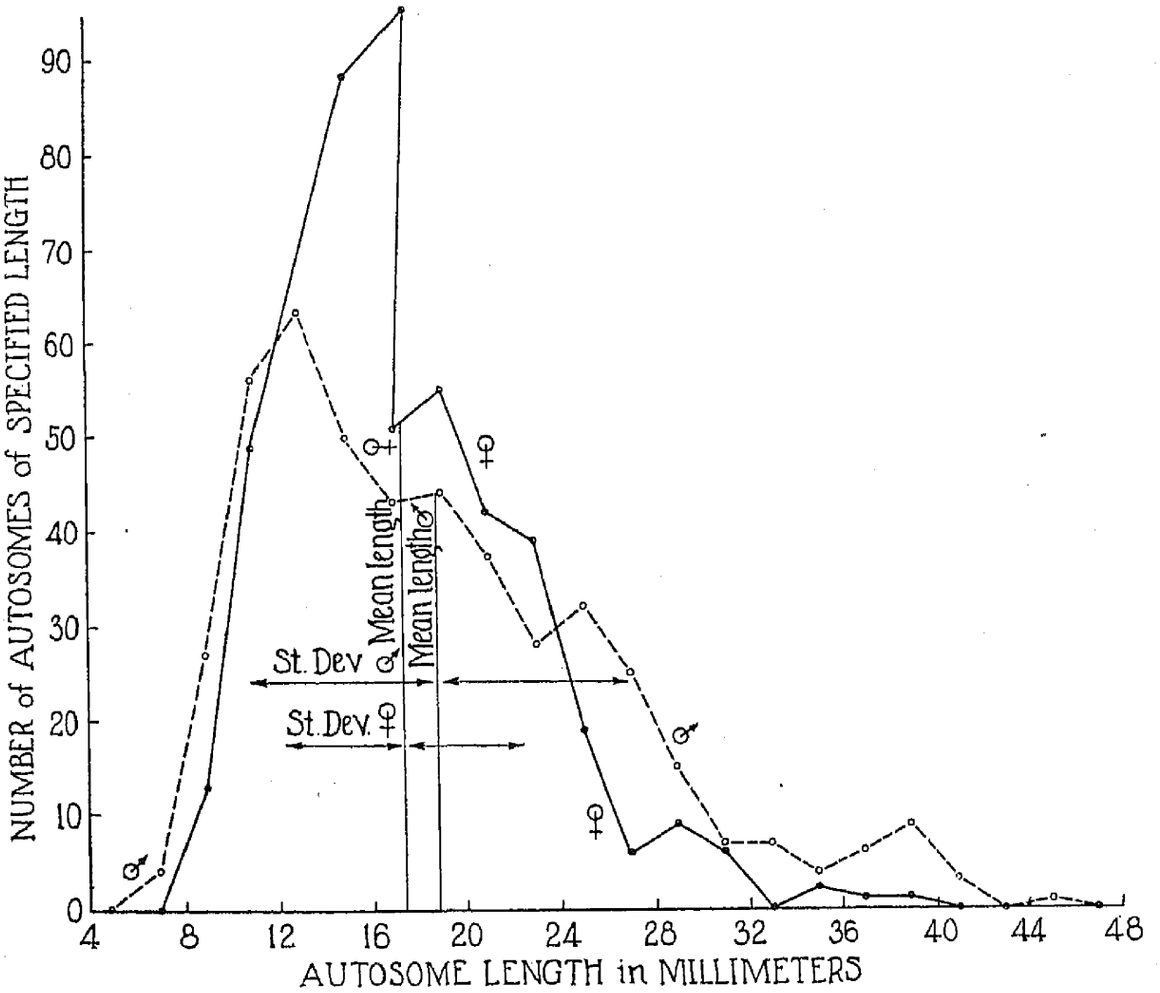


FIGURE 1.—Frequency distribution of autosome measurements in human embryo nuclei; all 46 autosomes in 10 nuclei of each sex.

It will be noted that the standard deviations are not significantly different in the two sexes when only one pair of autosomes is considered. When all twenty-three autosome pairs were studied (neglecting the XX and XY pairs) and the mean autosome lengths computed, the excess in length of male autosomes is seen to be much less than that shown in the case of the longest (first) pair. A difference of only 1.56 mm results. The probable error of the difference is .30 mm. Thus, though this difference is small it is also significant, being over five times its probable error. The frequency distributions of these measurements are given in table 3.

It is interesting to note, both from the frequency distributions and the computed standard deviations, that, when all twenty-three pairs of autosomes are considered, the male autosomes show a much greater variability than the female autosomes. The difference between the standard devia-

TABLE 3

Frequency distributions of autosome measurements

AUTOSOME LENGTH IN MM	ALL THE AUTOSOMES—10 NUCLEI				LONGEST PAIR ONLY			
	Human embryos		Rat embryos		100 nuclei from one individual rat embryo		10 nuclei from each of 10 rat embryos	
	Male	Female	Male	Female	Male	Female	Male	Female
4.0 to 5.9	..	..	2	..	..	..	..	..
6–	4	..	5	1	..	..	..	..
8–	27	13	13	8	..	..	..	..
10–	56	49	41	49	..	..	..	..
12–	63	79	77	71	..	..	..	..
14–	50	88	82	78	..	..	..	..
16–	43	51	82	75	..	..	..	..
18–	44	55	81	71	..	..	..	..
20–	37	42	48	51	..	..	..	..
22–	28	39	52	53	..	2	..	..
24–	32	19	26	44	..	10	..	1
26–	25	6	15	34	4	17	..	3
28–	15	9	13	18	3	28	2	20
30–	7	6	15	19	15	37	8	29
32–	7	..	6	12	22	41	7	56
34–	4	2	9	6	29	25	18	29
36–	6	1	9	3	43	22	32	30
38–	8	1	3	4	22	11	43	17
40–	3	..	2	..	19	4	24	7
42–	..	..	6	..	20	3	24	5
44–	1	..	4	1	9	..	17	2
46–	..	..	3	2	6	..	10	1
48–	..	..	..	..	7	..	3	..
50–	..	..	2	..	..	..	7	..
52–	..	..	2	..	..	..	1	..
54–	..	..	..	..	1	..	1	..
56–	..	..	1	..	..	..	..	..
58–	..	..	1	..	..	..	..	..
60–	..	..	..	..	..	..	3	..
Total	460	460	600	600	200	200	200	200

tions is 2.29 ± .21 mm so that the difference is about ten times the probable error of the difference. The same sex difference in variability is shown by the coefficients of variation, which are, respectively, 39.70 ± 1.02 mm and $30.01 \pm .72$ mm.

This greater variability is, in fact, so great that the sex difference in mean autosome length is actually reversed when the shortest pair of autosomes is considered. This fact is, of course, indicated by the much

smaller sex difference in mean autosome length which was noted when all twenty-three pairs of autosomes were averaged, as compared with the sex difference when the longest pair only was considered.

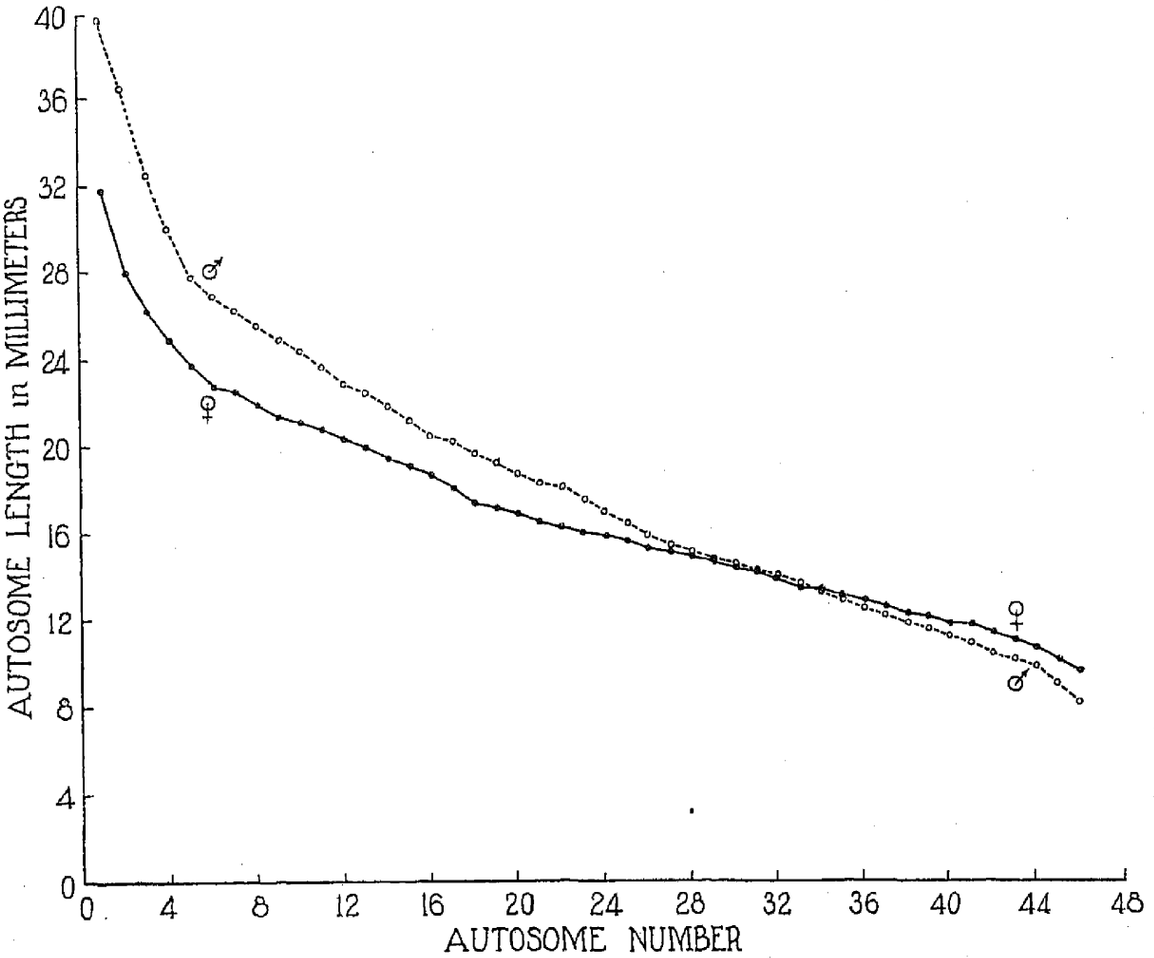


FIGURE 2.—Average lengths of successive autosomes in 10 nuclei of each sex—human embryo.

It thus becomes of interest to consider the smallest pair only. These data also are presented in table 1 and the constants in table 2. In this case the autosome lengths in the female *exceeded* those in the male by 1.31 ±.17 mm. The difference thus indicates that in this male embryo the shortest autosomes were significantly shorter than the shortest autosomes of the female embryo.

Since the standard deviations are again not significantly different, the greater variability of the lengths of the autosomes of the male indicates a greater variation or range in length of the autosomes within a single nucleus, and not a greater variation between lengths of corresponding autosomes in different nuclei. Table 4, showing the averages and standard deviations of the twenty-three pairs in each of the ten nuclei of each sex, is presented to corroborate these points. The standard deviations for the individual nuclei of the male range from 6.64 to 8.06 mm with a

value for the total distribution of 7.46±.17 mm; those of the female range from 4.03 to 5.69 mm, with a value for the total distribution of 5.17±.11 mm. The difference in standard deviations is thus 2.29±.20 mm. The difference in variability is therefore highly significant. Figure 1, plotting the frequency distributions, means, and standard deviations of the autosome lengths and figure 2, plotting the average lengths of the successive autosomes, show these sex differences graphically.

TABLE 4

Average of all 46 autosomes in each individual nucleus (in mm) in ten nuclei of human embryos of each sex.

NUCLEUS	MALE		FEMALE	
	Mean	Standard deviation	Mean	Standard deviation
1.	18.80±.69	6.94±.49	15.83±.40	4.03±.28
2.	17.57±.70	7.02±.49	17.39±.54	5.46±.38
3.	17.48±.76	7.62±.54	16.65±.49	4.89±.34
4.	17.52±.66	6.64±.47	16.09±.51	5.17±.36
5.	19.83±.70	7.08±.50	18.17±.55	5.57±.39
6.	20.17±.73	7.32±.51	16.70±.55	5.55±.39
7.	17.61±.80	8.06±.57	17.17±.47	4.68±.33
8.	21.17±.80	8.04±.57	18.09±.57	5.69±.40
9.	18.74±.68	6.88±.48	18.57±.54	5.40±.38
10.	18.74±.79	7.94±.56	17.61±.42	4.23±.30
Average	18.79±.23	7.46±.17	17.23±.16	5.17±.11

We were interested in seeing whether this sex difference in chromosome lengths, so evident in embryonic tissue, is demonstrable in the tissue of adults. Unfortunately tissue growth in the male, save in the testis, (spermatogenesis), consists merely in the relatively slight normal reparative processes seen, for instance, in the epithelia of the skin and gut. We hence sought neoplastic growths in boys or men and finally secured a labial carcinoma in a man of 55 years, where mitotic division was rife. The use of this material is unfortunately open to the charge of "embryonic character" of neoplastic growth. In the case of the female, normal reparative processes in the endrometrium of early post menstruum furnished abundant instances and stages of dividing cells.

Measurements were made of chromosome lengths in ten nuclei of each sex. Here again the longest autosome pair of the male exceeded that of the female by ten times the probable error of the difference (table 6). When the lengths of the twenty-three pairs of autosomes were averaged,

TABLE 5

Individual measurements of extreme autosomes (in mm) in ten nuclei of human adults of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	40.4	37.1	27.2	26.3	7.0	10.0	6.7	9.3
2.	47.9	38.1	34.3	33.0	9.0	9.3	10.1	10.3
3.	37.2	37.0	30.1	25.8	10.5	11.0	9.1	9.3
4.	38.0	31.0	28.8	25.3	10.0	10.5	11.1	12.0
5.	39.0	34.5	28.9	28.3	13.1	14.8	10.0	10.3
6.	36.0	30.3	30.6	30.5	10.5	11.5	8.5	9.2
7.	50.0	44.8	31.6	31.5	9.2	10.2	11.0	11.4
8.	42.0	40.5	36.8	32.8	10.0	12.1	8.5	9.5
9.	37.3	33.5	24.0	22.7	11.4	12.0	7.5	7.5
10.	44.3	44.0	32.3	29.2	8.9	10.2	8.3	8.9
Average	41.2	37.1	30.5	28.5	10.0	11.2	9.3	9.8

TABLE 6

Human adult (10 nuclei of each sex, measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	39.15±.76	5.07±.54
Longest pair ♀	29.49±.54	3.58±.38
Difference ♂ − ♀	9.66±.93	1.49±.66
Average of all 46 autosomes ♂	20.12±.22	7.15±.16
Average of all 46 autosomes ♀	16.46±.16	5.24±.12
Difference ♂ − ♀	3.66±.27	1.91±.20
Shortest pair ♂	10.56±.25	1.63±.17
Shortest pair ♀	9.53±.18	1.21±.13
Difference ♂ − ♀	1.03±.31	.42±.21

the difference between male and female (3.66 mm), though smaller in the case with the longest pair only (9.66 mm), is nevertheless, also ten times its probable error. Even the shortest autosome pair in these adult autosomes was longer in the male than the female, though the sex difference here is by no means as great as with the longest pair. It will be remembered that in the embryonic mitoses the shortest male autosomes were shorter than the female ones. The mensurations of adult chromosomes showed, as did the embryonic, a much greater variability in male autosome lengths within a given nucleus as compared with female, whether measured by standard deviations or by the coefficients of variation. These measure-

ments thus furnish a confirmation of Darwin's belief in a greater variability in the male as a general law.

TABLE 7

Average of all 46 autosomes in each individual nucleus (in mm) in ten nuclei of human adults of each sex.

NUCLEUS	MALE		FEMALE	
	Mean	Standard deviation	Mean	Standard deviation
1.	18.61±.65	6.51±.46	16.39±.46	4.58±.32
2.	19.26±.79	7.97±.56	17.30±.65	6.54±.46
3.	19.87±.61	6.15±.43	15.61±.47	4.77±.34
4.	20.04±.69	6.92±.49	17.30±.43	4.29±.30
5.	20.52±.48	4.85±.34	17.52±.43	4.34±.31
6.	18.96±.55	5.49±.39	14.13±.46	4.60±.32
7.	21.39±.89	8.92±.63	18.09±.48	4.86±.34
8.	21.30±.74	7.47±.53	17.74±.58	5.87±.41
9.	20.96±.68	6.85±.48	14.30±.44	4.38±.31
10.	20.30±.86	8.65±.61	16.17±.58	5.85±.41
Average	20.12±.22	7.15±.16	16.46±.16	5.24±.12

Though we had taken all of the precautions possible to insure reliability in our conclusion of increased length of the longest male autosomes, the number of actually different animals studied was so small that we sought added evidence through the study of another mammalian form (the rat) and through a more rigorous control of all the factors influencing so delicate a task.

A pregnant rat was chloroformed on the twentieth day of gestation. The successive uterine enlargements were rapidly and deftly opened with fine tweezers and scissors, the embryos being instantly plunged under Bouin's fluid at 39°C. In each case the central body wall was cut widely open, enabling an immediate view of the gonads and diagnosis of sex to be made. We took care to secure identity in the fixation, dehydration, and all technical procedures involved in sectioning and staining the male and female litter mate embryos. Here again, in each sex, we delineated all the chromosomes in ten prophase mesenchyme nuclei located near the skin ectoderm and ideally fixed. As one of us has discovered (SWEZY, 1927), the hybrid rat of our colony possesses both sixty-two and forty-two chromosomes instead of the single count of forty-two which, in agreement with PAINTER (1927), we find the albino possesses. The rat chromosomes were all magnified 8400 diameters for measurement. The data are presented in table 8 and the constants in table 9.

TABLE 8

Individual measurements (×8400) of extreme autosomes (in mm) in ten nuclei from rat embryos of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	44.8	42.0	32.7	32.2	8.3	8.5	10.7	10.7
2.	46.7	41.1	44.6	39.6	8.0	8.2	11.0	11.1
3.	39.5	39.3	35.2	33.7	7.3	7.3	8.5	9.5
4.	57.8	45.8	46.5	37.8	6.5	10.7	8.2	10.0
5.	59.3	51.6	31.3	30.6	10.7	11.2	9.7	10.0
6.	52.1	46.5	32.8	32.5	10.7	10.8	7.5	8.8
7.	44.0	42.2	36.1	33.6	10.1	10.6	10.7	10.8
8.	42.3	36.9	33.1	34.1	4.5	7.6	10.6	10.6
9.	45.3	43.9	47.5	38.3	5.0	9.0	10.0	10.0
10.	53.2	42.0	36.0	35.8	6.0	9.4	8.7	10.2
Average	48.5	43.1	37.6	34.8	7.1	9.4	9.5	10.2

TABLE 9

Rat embryo (10 nuclei, measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	45.81± .89	5.92±.63
Longest pair ♀	36.20± .72	4.80±.51
Difference ♂ − ♀	9.61±1.14	1.12±.81
Average of all 60 autosomes ♂	19.71± .22	8.16±.16
Average of all 60 autosomes ♀	19.61± .18	6.38±.12
Difference ♂ − ♀	.10± .28	1.78±.20
Shortest pair ♂	8.24± .45	2.98±.32
Shortest pair ♀	9.84± .18	1.22±.13
Difference ♂ − ♀	1 .60± .49	1.76±.34
	Rat Embryo (100 nuclei from one individual)	
Longest pair ♂	37.87± .24	5.03±.17
Longest pair ♀	32.39± .20	4.11±.14
Difference ♂ − ♀	5.48± .31	.92±.22
	Rat Embryo (10 nuclei from each of ten different individuals)	
Longest pair ♂	40.30± .26	5.45±.16
Longest pair ♀	34.02± .18	3.82±.13
Difference ♂ − ♀	6.28± .32	1.63±.21

Here again a clear difference exists in the case of males and females in the length of the first autosome pair, the difference between the means being 9.61 mm with a probable error of 1.14 mm. The difference is thus approximately nine times the probable error of the difference. The difference between the standard deviations is insignificant just as in the human nuclei when only one pair of autosomes is considered.

In the case of the rat, when the entire sixty or forty chromosomes were studied (omitting the XX and XY pairs) and a grand mean thus established, the superiority in length of the male autosomes practically completely disappears. The difference between the means is then merely .10 mm, with a probable error of .28. Thus, in the rat, as in man, the sex difference in autosome length is reversed in the smaller pairs of autosomes. The difference between the standard deviations is $1.78 \pm .20$ mm. The autosomes of the male rat, like those of man, thus show a greater variability than those of the female. The coefficients of variation are here $41.40 \pm .93$ mm and $32.53 \pm .69$ mm.

Computing the constants for the shortest pair only, the actual excess in length of the *female* autosomes is found to be $1.60 \pm .49$ mm. The difference in standard deviations is in this case significant, the first case in which a significant sex difference in variability had been found when only one pair of homologous autosomes in different nuclei is considered. The difference is $1.76 \pm .34$ mm.

To dissipate any lurking suspicion that we had consciously or unconsciously *selected* male prophase nuclei with longer autosomes than in the female, we decided to study a hundred nuclei from each sex, considering the two longest autosomes only. It does not seem to us remotely possible that biased choice could have been exercised by us in such an extensive record. The results of these measurements gave us a difference between the average length of the male and the female first autosome pair of 5.48 mm, with a probable error of only .31 mm. The difference between these means is highly significant, being approximately eighteen times its probable error. The frequency distributions of these measurements are given in table 3. The standard deviations are here again significantly different.

As a fifth test of this sex difference, it was thought desirable to secure corresponding measurements in a large number of different individuals. Therefore the longest pair of autosomes was measured in 10 nuclei from each of 10 different rat embryos of each sex. The averages for the different individuals are listed in table 10; the constants computed from the entire series, in table 9; and the frequency distributions of the individual measurements in table 3. This series of measurements again shows the

same sex difference, the longest autosomes of the male being significantly longer than the longest autosomes of the female. The difference in this case is $6.28 \pm .32$ mm, which is nearly twenty times the probable error of the difference. The difference is nearly ten times the probable error of the difference when the average for each embryo is treated as a single observation. (It will be noted that the individuals from which the 100

TABLE 10

Average lengths of longest pair of autosomes in ten different rat embryos of each sex.

EMBRYO NUMBER	AVERAGE OF 10 NUCLEI OF EACH EMBRYO			
	Male		Female	
1	39.8	35.4	39.4	34.9
2	50.1	44.5	40.0	35.1
3	39.6	37.4	35.8	32.5
4	41.7	38.1	35.0	32.1
5	39.3	36.7	33.1	31.2
6	43.1	38.4	35.0	30.9
7	46.0	42.6	34.0	31.3
8	39.8	35.2	35.0	32.8
9	41.9	38.2	32.9	30.9
10	41.7	36.2	35.8	32.8
Grand Average	42.3	38.3	35.6	32.5

TABLE 11

Rat Embryo (10 nuclei, measurements (×8400) in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	$45.81 \pm .89$	$5.92 \pm .63$
Longest pair ♀	$36.20 \pm .72$	$4.70 \pm .51$
Difference ♂ − ♀	9.61 ± 1.14	$1.12 \pm .81$
Average of all 60 autosomes ♂	$19.71 \pm .22$	$8.16 \pm .16$
Average of all 60 autosomes ♀	$19.61 \pm .18$	$6.38 \pm .12$
Difference ♂ − ♀	$.10 \pm .28$	$1.78 \pm .20$
Shortest pair ♂	$8.24 \pm .45$	$2.98 \pm .32$
Shortest pair ♀	$9.84 \pm .18$	$1.22 \pm .13$
Difference ♂ − ♀	$1.60 \pm .49$	$1.76 \pm .34$
	Rat Embryo (100 nuclei)	
Longest pair ♂	$37.87 \pm .24$	$5.03 \pm .17$
Longest pair ♀	$32.39 \pm .20$	$4.11 \pm .14$
Difference ♂ − ♀	$5.48 \pm .31$	$.92 \pm .22$

nuclei were measured happened to be near the lower end of the range of variation displayed by the 10 different embryos in this last series, while the individuals from which the first 10 nuclei were measured happened to be near the upper end of the range.)

Thus in five independent series of measurements, comprising the measurements of all the autosomes of ten nuclei of one individual of each sex in human embryo, human adults, and rat embryos, and the measurements of the longest pair of autosomes in one hundred nuclei from one rat embryo of each sex, and from ten nuclei from each of ten rat embryos of each sex, consistent and highly significant sex differences were found. The same sex difference in length of the longest autosomes is shown but not commented upon in part of the figures in PAINTER'S (1924) plate of the chromosomes of the monkey.

A study is being made of the reduction divisions in spermatogenesis to see whether this sex difference is due to a selective segregation of the chromosomes in the spermatids. This will be reported upon at a later date.

SUMMARY

1. In man and in the rat a significant and consistent sex difference occurs in the length of the autosomes of the somatic cells of both the embryo and adult.

2. The longest pair of autosomes in the male is longer than the longest pair in the female. This difference may be related to the greater body size generally found in the male.

3. Males also show a greater variation in the length of the autosomes within an individual nucleus. This difference could not be predicted from the greater body size of the male.

4. Thus, in addition to their possession of different chromosomes—the so-called sex determining chromosomes, the XX and XY pairs— male and females differ in the character (length) of the autosomes. This difference occurs in the early embryo and persists in the adult. It probably appears at the time of fertilization and can be looked upon as a "secondary" sex character inpressed from the beginning upon every cell in the body

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A CONSIDERATION OF VARIEGATION

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The segregation of albino or chlorophyll deficient seedlings in a pedigree propagated by self-fertilization is due either to a definite genetic factor or factors according to DEMEREC (1923) in maize, HALLQVIST (1926) in barley, etc., or to the abnormality of the plastids themselves according to CORRENS (1909) in Mirabilis, BAUR (1909) in Pelargonium, ANDERSON (1923) in maize, CLAUSEN (1927) in Viola, etc. In the former cases, the albino segregates occur in one-fourth, seven-sixteenths, one-sixteenth and so on, according to the number and behavior of the factors by which the deficiency of chlorophyll is manifested, while in the latter their segregating proportion is quite irregular, varying according to the degree of variegation on the parental plants from which the seeds were collected, or, in other words, the abnormal plastids conduct themselves independently of the genetic factors, their transmission therefore being non-Mendelian. Professor Sô (1921) studying the genetics of variegated barley made its peculiar behavior clear. His conclusions published in the Japanese language induced me to write this paper, in which I will give a critical discussion of the complicated heredity of some variegated plants in the light of Professor Sô's work and of analogous cases from observations made by myself.

I

Before entering into the main discussion, I will describe the results gained in my provisional studies on the variegated barley and rice. Once there came to my attention a case of the ordinary non-Mendelian variegation in barley. In 1923 I found a plant with coarse yellow stripes distributed over some leaves and an ear, and transplanted this to our experimental field to study its genetic nature. Owing to its poor growth and to transplantation at the flowering time, the seeds obtained were not numerous. From the green ears I raised 38 green seedlings, on which later no yellow stripes developed, and from the yellow part of a variegated ear 8 seedlings were obtained, all yellow, which died a few weeks after their germination.

Variegated seedlings are often found in rice, *Oryza sativa*, though they are rare in barley. In most cases the variegation occurs in coarse yellow stripes and is apt frequently to disappear in later growth. I collected the

seeds from a variegated rice plant and obtained 155 seedlings containing 48 yellows or 30.97 percent, the others being pure green, excepting one seedling which had a few yellow stripes on the lower leaf. The variegation on this specimen, however, completely disappeared in the later leaves and pure green ears only were produced. Althouh I did not carry out hybridization experiments with the green-and-yellow-variegated barley and rice, their non-Mendelain nature is certain from the abnormal segregation in their self-propagated progeny. The continuation of variegation of the ordinary non-Mendelian type by seeds is difficult in rice, and this is closely connected with its particularly coarse pattern of variegation. The cause of the variegated pattern, coarse or fine, of the non-Mendelian type was explained logically by WINGE (1919), who states in this connection: "Elementary mathematics will tell us that cells with a small number of green and white plastids will, by constant bisection in the course of a few cell generations, possibly even on the first division, give off both purely albinotic and self-colored green cells in the descendance, and only a very few of a mixed character, later none at all—whereas cells with but a slightly (not greatly) superior number of green and white plastids will only after a several cell generations, when the embryo has reached a considerable size, give off albinotic and self-colored green cells, and will never in the course of the ontogenesis lack cells with mixed contents" (p. 15). The different distribution of the abnormal plastids will give the corresponding difference in the proportion of variegateds in the offspring. Barley and rice stand at one extreme in regard to the small number of plastids contained in a cell, among the known non-Mendelian variegated plants. If this is the case the variegation should give a coarse pattern and the specimens should give a progeny containing green and yellow seedlings with a few or no variegateds, as actually recorded. On reading the subsequent sections we must bear in mind that the sorting out of the homogeneous plastids will occur very easily in barley and rice in the course of cell.multiplication.

II

Among the specimens of barley cultivated in our College there is a variegated variety named "Okina" (old man). The foliage and ears of this variety are variegated with fine white stripes, and its varietal name came from its ears bearing some long white awns, reminding one of an old-man's white hair. The progeny of this variety contains some white seedlings, which die in a few weeks after germination when the food stored up in the endosperm is consumed. The proportion of albino

seedlings varies to some extent according to different samplings, though, on the whole, it is almost fixed. The seedlings, other than albinos, invariably grow up to be variegated. Observation on fully grown plants shows constancy in these respects. The variegated characteristic is segregated as a Mendelian recessive to the normal green. Sô (1921) studied thoroughly the genetic behavior of the variegation of this variety, and his main results, freely translated from the Japanese, are as follows:

1. The albinism is transmitted only maternally, the pollen having no immediate connection with it.
2. Variegation behaves as a recessive to green.
3. In F_2 and later generations, the segregation of variegation occurs in a mononhybrid ratio.
4. In crossing experiments, the pollen of pure white glumes is not different in its effect from that of green ones of the variegated ear.

On the basis of these experimental facts he developed his theory to account for the special behavior of the variegated barley in inheritance. The variegated character is transmitted as a recessive genetic factor, but it is not a pattern factor in a strict sense, because the white parts or stripes are due to the distribution of white plastids which have changed their quality permanently. The variegation is, therefore, produced by a factor which alters at times the essential quality of the plastids from normal green to colorless. The original difference between green and variegated barley lies in their allelomorphs for the stability of the plastid, and the variegation is due to the development of white stripes, owing to the distribution of the white plastids controlled no longer by genetic factors. To be more particular, let us designate the allelomorphs concerning the plastid mutability by G (dominant) and G' (recessive). Normal green barley is GG, and the variegated barley $G'G'$. The plastids of both are originally quite normal in their essential quality. Each of them is designated by g. It is assumed that in the presence of $G'G'$ (double recessive) some of the green plastids mutate at times to white plastids (w). The plastids of self-green barley are all g, while those of the variegated barley consist of g and w. The former (g) may subsequently be altered in the presence of the factors $G'G'$ to become white.

The plastid mutation, that is sometimes observed in various plants, is due in almost all cases to sporadic events and does not seem to be a habitual phenomenon. In the "Okina"-barley, however, the plastid mutation repeats itself so frequently in the course of the development of the plant body that not a single individual or even a leaf or an ear,

which is free from the variability or self-green, is produced. The variegated segregates appear in a recessive proportion from green hybrid plants. According to Sô's experiments, the white plastids are transmitted maternally. Under such circumstances, the reciprocal crossings of the normal and variegated varieties give partly different results in F_1, while practically the same in the later generations. Figure 1 is a diagrammatic

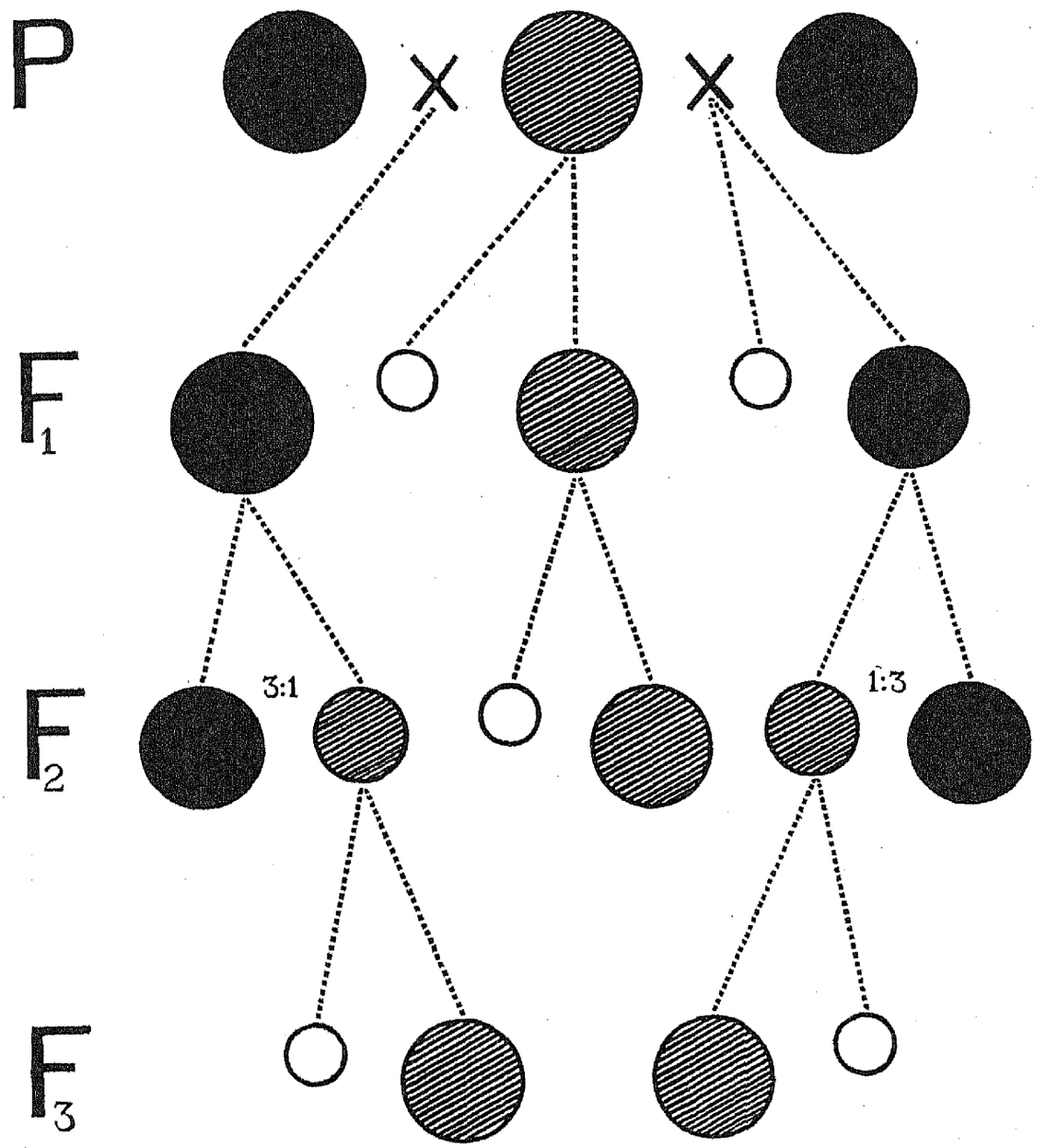


FIGURE 1.—Showing the heredity of variegated barley of the "special" type. The black circle indicates the green plants, the barred the variegated and the blank circle the albinotic. The size of the circles roughly represents the proportion of the respective segregates in a pedigree. The left group of F_1, F_2 and F_3 shows the results of cross-breeding of green (♀)×variegated (♂), those in the center the inbreeding of the variegated, and those at the right the cross-breeding of variegated (♀)×green (♂).

summary of inbreeding and cross-breeding of the "Okina"-barley, drawn from the results obtained by Sô. In barley the chance that white plastids and green ones are present in an egg-cell of a variegated plant may not be frequent, because the sorting out of homogeneous plastids will occur very easily in the foregoing cell-division, and yet the seedlings other than albinos are finely variegated, due to new plastid mutations occurring in their later embryonic stage. Sometimes the variegation of the seedlings is not clear, but successive observation make its existence manifest. The absence of pure white leaves and ears on the field-grown variegated barley, according to my opinion, may be regarded as due to the non-mutability of plastids in cells of the primitive ontogenic stage, including the rudiments of leaves and ears. As a result of this, there is no chance of obtaining pure white leaves or ears developed from white cells forming their rudiments. The absence of plastid mutability seems also to be the case in gametogenesis as well as in later somatic divisions. The plastid mutation occurs repeatedly during the development of the somatic tissues, excepting its very earlier and later times, and by the sorting out of the albinotic plastids the white stripes appear on the foliage. But owing to the non-mutability in the later somatogenesis the purity of cells for their plastids will be maintained. As a natural sequence we should obtain almost exclusively two kinds of gametes, the one containing all green plastids and the other all whites, by the variegated plants, in the same way as those of the ordinary non-Mendelain type. The data obtained by the crossing variegated (♀) × green (♂), which give rise to pure green and albino seedlings in F_1, comfirms this view. Practically, however, a few seedlings assuming a sectorial mosaic of white and variegated (or white and apparently green), though they rarely occur, are found among the progeny of variegated barley under my observation. According to my view, they must be regarded as developed from egg-cells containing green and white plastids together. If such seedlings are carefully cultivated we should obtain either white leaves or white ears, which are never found in the field-grown variegated barley in question, so far as our observation goes. Summing up, the plastid mutation period seems to begin at a late embryonic stage but ceases in later somatogenesis.

The variegation of the barley, therefore, is due to the distribution of a non-Mendelian albinotic character, but the causes of its repeated occurrence is due to a Mendelian factor. The genetics of this variegated barley is not comparable with the ordinary variegations studied in various plants, the case being "special" in its behavior. The variegation

in my yellow-striped barley was coarse, but that of "Okina"-barley is relatively fine; the difference is accounted for by the different genetic conditions by which the variegation appears.

III

Recently KONDÔ, TAKEDA and FUJOMOTO (1927)[1] have published a paper on the genetics of variegated rice from pedigree cultures of this plant. Their green-and-white-variegated races of rice are grouped under two classes in regard to hereditary type; in one the variegateds reappear in great numbers in the progeny, and in the other practically no variegateds are present but segregation produces green and white seedlings. The latter may be identified with that described in Section I. Among their experiments, A and B are treated principally as one and the same, therefore I will consider them together in the following discussion. In these experiments they obtained 64.5 percent of albino seedlings in the progenies of the variegated plants, the others being apparently green. When these green seedlings are transplanted into the paddy-field in order to allow their later growth, the majority grow up to be variegated and a few remain normal. The average proportion of white, variegated and green plants are 64.5 percent, 30.8 percent and 4.8 percent respectively. The albinos die soon after their germination. The green plants, which appear in the progeny of the variegated rice, breed invariably true to the type, while the variegated sisters repeat the occurrence of three forms in their offspring. With such an hereditary nature we cannot think of Mendelian segregation or ever-occurring factorial mutation. KONDÔ and his collaborators also made a hybridization experiment. They made a cross of variegated by green, from which they obtained variegated F_1 plants and bred an F_2 consisting 77.6 percent of albinos, 18.0 percent of variegateds and 4.4 percent of greens. This result is principally the same as that obtained by inbreeding the variegated rice in question. In the reciprocal crossing, they obtained only green F_1 plants. Therefore, the heredity of variegation in rice is maternal. The experiment C made by them gave quite a different result, though practically the same as in my case. My view of this is that it is due to ordinary non-Mendelian inheritance. KONDÔ and others regarded this and the former as equally ordinary non-Mendelian cases and their quantitative difference in the progeny was attributed to the occurrence of sterile flowers. The experiment C was made with variegated rice which was

[1] Their Japanese paper was published in the Journal of Scientific Agricultural Society, No. 277 pp. 443–462, 1925.

semi-sterile at the same time. The albinos obtained from four variegated plants were only 4.9 percent of their offspring, the others being pure green without exceptions. In their opinion this result is explained as follows:

"Vielleicht gibt es sehr viele Blüten, welche die Eigenschaften der Albino und zugleich die der Sterilität besitzen, was die Minderung der Prozentzahl der Albino bei Versuch C verursachen könnte" (loc. cit., p. 301).

The principal diversity of two variegated types of rice lies in the considerable difference of the frequency in appearance of variegated individuals in the succeeding generations. The genetic type of the variegation treated by KONDÔ and his collaborators in their experiments A and B does not seem to be thoroughly clear from the theory proposed by them. In my opinion, the case is complicated by the habitual occurrence of plastid mutation as shown in barley, therefore we meet with another not common case of non-Mendelian inheritance. The "unusual" type of variegation of rice has no connection with any factors contained in the chromosomes in its development as well as in its inheritance and the specimens carry quite normal genetic chlorophyll factors. The qualitative peculiarity of the cell-component of the variegateds in rice is due to the particular nature of the plastids themselves. The plastids may be normally green, containing ample chlorophyll, but it is their qualitative peculiarity that they are apt to alter themselves into white plastids. The habitual plastid mutability of this case is caused by the nature of the plastids themselves and this gives a contrast to the "special" form of the variegated barley due to the manifestation of a definite genetic fator, which stimulates the frequent occurrence of the plastid mutation. If we represent the abnormal green plastid by the symbol g', an inconstant state, it at times transforms itself into w, which has an analogy to the white plastid of the "special" form of barley in its hereditary quality. As a result of the frequent occurrence of $g' \rightarrow w$, the white stripes will appear on the foliage and ears by the sorting out of cells, which contain only white plastids, and their multiplication in the course of plant development. These cells in turn produce albino seedlings in their offspring. The remarkable divergence in the proportions of albino seedlings between rice and barley, in which the respective average counts show about 65 percent and 7 percent, will roughly indicate the difference in the frequency of plastid mutation occurring in the mother plants. The absence of all white leaves and ears is quite comparable with that observed in the variegated barley of the "special" type. The frequency of the segregation of albino seedlings is rather fixed,

though it varies to some extent in different samplings, and this is owing to the fact that all ears are finely variegated, due to the frequent occurrence of plastid mutation. If the plastid mutation occurs also in the early ontogeny of an individual or the rudiment of an ear or a leaf and the frequency of alternation is very low or entirely absent, we should have no such rather fixed proportion in the segregation of albinos. The white stripes run longitudinally on leaves and stems extending to the ears; this tells the direction of cell division in the ontogeny of Gramineae; that is longitudinal divisions generally take place early, while the transverse divisions are on the whole late. Such a mode of organ formation will limit the extension of the breadth of white stripes, as in the cells forming rudiments of leaves and ears the plastid mutation does not generally occur, but naturally it helps to regulate the average proportion of the segregation of albino seedlings.

The appearance of green plants among the progeny of the variegated rice of the "unusual" form is, in my opinion, in accordance with the plastid mutation of $g' \rightarrow g$, the latter being the non-mutable state. The low proportion of the green offspring seems to be partly connected with the small distribution of green-glumed flowers in the panicle of the variegated rice, the fact suggesting a limited period at which the plastid mutation of $g' \rightarrow g$ occurs partly due to the low frequency of its mutability, though the occurrence appears to be habitual. Among the pedigrees of KONDÔ and others, the family E/1/2/3/4 contained 22.5 percent of green individuals; this seems to be due to the qualitative alteration of a plastid or plastids in relatively early plant growth.

According to KONDÔ and his collaborators the original "unusual" variegateds, with which they carried on their experiments above cited, made their appearance as seed variation. The existence of such plants among pure green pedigrees may on the other hand be attributed to simultaneous or successive alternation of the plastids contained in an egg-cell. Supposing that the plastids of one egg-cell are all transformed from g to g', the cell could develop into a variegated plant after fertilization, since the pollen contributes no plastids to the egg in this species.

Before the publication of KONDÔ, TAKEDA and FUJIMOTO's paper, TAKEZAKI (1922) discussed the genetics of variegated rice, the transmission of which can be identified with the "unusual" form of the former investigators. Strictly speaking, TAKEZAKI's result was verified by them. TAKEZAKI interprets his case in the ordinary non-Mendelian mode of inheritance, but it is equally due to the peculiar nature of plastids contained in his variegated rice. As regards his experiments I will especially

cite the results obtained by crossings, which were made in both reciprocal ways and traced up to F_2. The F_1 plants obtained by the cross, variegated (♀)× green (♂), consisted mostly of variegateds, the rest, 20 percent, being albinos. At the seedling observation of F_2 TAKEZAKI found 42 percent albinos, the others apparently green, which later turned into variegated plants containing 7.15 percent of green sisters.

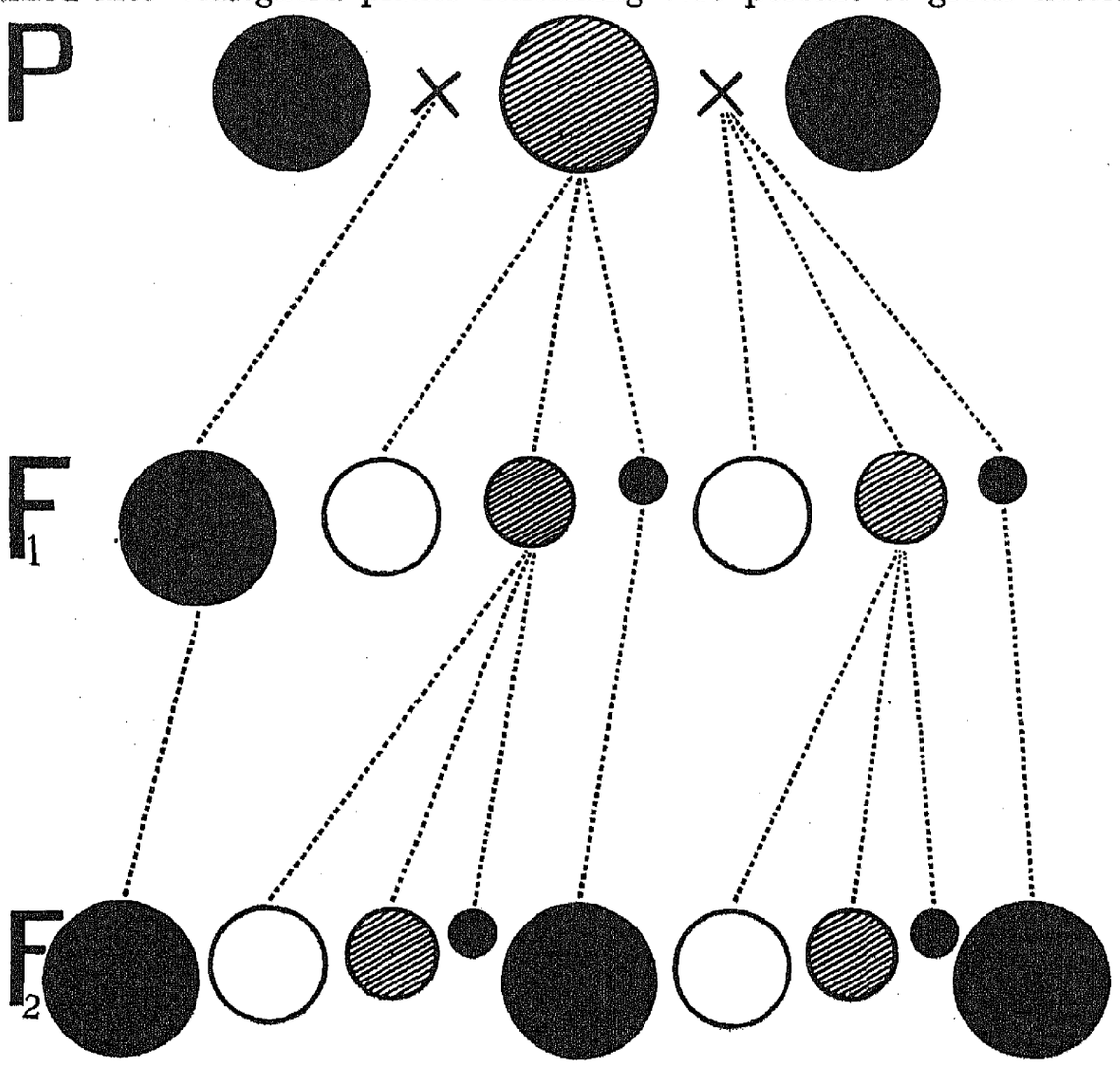


FIGURE 2.—This diagram as in figure 1 shows the hereditary mode of the variegated rice of the "unusual" type.

In the reciprocal crossing he obtained pure green F_1 and F_2. With his experiments the evidence of the maternal inheritance is quite clear. The genetic nature of the "unusual"-typed variegated rice is shown graphically in figure 2, which is drawn in conformity with the theoretical explanation based on data obtained by TAKEZAKI, KONDÔ, etc.

IV

The non-Mendelian variegation is generally due to a mixture of abnormal plastids which have changed from the normal. The sporadic

plastid mutation occurs at times in various plants. In these cases, the green branches are often produced by the sorting out of green plastids in cell multiplication. If the plastid mutation is frequently repeated the green branches cannot remain as such owing to the successive occurrence of albinotic plastids, as demonstrated by barley and rice. I have called such cases "special" or "unusual" in contradistinction to the ordinary non-Mendelian variegation. There are several variegated plants which can be considered as analogous to the not-common class. The following garden varieties of coniferous plants came under my observation, and I have met with several similar cases in the other variegated plants, a discussion of which will be left for another opportunity.

Chamaecyparis obtusa, variegated.

The variegation occurs in creamy yellow color. The development of the yellow parts on the variegated plant is abnormal, resulting in greatly contracted branchlets.

Ch. obtusa var. *breviramea*, variegated (Jap. name "Shônosuke-Hiba").

Greenish, creamy yellow variegation occurs on the foliage. Yellow branchlets of abnormal development are frequently produced. In this variety pure green branches are produced, which occurrence, however, cannot be comparable with that of yellow branches in frequency.

Ch. pisifera var. *breviramea*, variegated (Jap. name "Wakoku-Hiba").

This form has yellow patches on the foliage. The yellow parts have an arrested growth with relatively massive branchlets. Sometimes pure green branches appear on this variety.

Ch. pisifera var. *plumosa*, variegated.

White branchlets occur here and there on the plant. The variety seems to be rare in our gardens, so far as my observations go. I observed three individuals of this form, each growing about 1.5 metres high, but found no green branches. In another form, white branchlets occur in a pepper-and-salt pattern among the green foliage, with green sports appearing at intervals. The foliage of the latter variegated form, however, is somewhat contracted and has a delicate appearance, which distinguishes it from the former.

Ch. pisifera var. *filifera*, variegated.

The variegation occurs in yellow color. So far as my observation went, no green sports were found.

Juniperus chinensis, variegated.

A form with white variegation is found in the "Byakushin" type, in which needle-like foliage occurs abundantly. The other forms belonging to the "Ibuki" type, in which scale foliage occurs abundantly, are variegated with yellow or creamy white. The yellow or white branchlets are found very often in these varieties, probably in the most frequency among the variegated forms of the coniferous plants listed here. The green branches on these "Ibuki" forms are due to bud-sports, but not to the sorting out of the homogeneous green plastids, as is the case in the yellow or white branchlets. The green branches make usually very vigorous growth. In the yellow-

variegated form the yellow branchlets make somewhat strong growth in size compared with those of the white-variegated form, and the golden patches on the foliage is very evident by their frequent occurrences.

J. chinensis var. *procumbens*, variegated.

The variegation occurs in white, like that of the "Byakushin" type above listed, but the occurrence of white branches in the latter form is somewhat lower in frequency. In these two forms no green sports occur, so far as my observations go.

In these plants, the variegation is very evident, without any intergradation of color. The patch-work is generally maintained throughout the plant growth. Since the white or yellow patches occur in a coarse degree the vegetative segregation of abnormal plastids at cell multiplication must easily take place, resulting in yellow or white branchlets. If the variegation is due to the mixture of abnormal plastids and the plastid mutation is not repeated, the segregation of the green branches should be very frequent, though it never occurs by such a way in actual cases. Hence I consider them as analogous to the so-called "special" or "unusual" genetic form of variegation, in which albinotic plastids occur repeatedly by mutation.

As I did not raise the progeny of these variegated plants or hybridize them with their respective normal greens, I cannot draw a conclusion as to whether the cases are of the Hordeum or of the Oryza or of any other type. Some variegated plants shown in the list, however, sometimes bear green branches, which are considered to be produced by vegetative variation instead of segregation, as they are hardly comparable with white branches in frequency of their occurrence. The green branches make vigorous growth and become permanently green.

An interesting fact which came to my attention is the characteristic of the green reverting branches occurring on a white-variegated form of *Chamaecyparis pisifera* var. *plumosa*. The green sporting branches do not have a foliage habit quite similar to the variegated form, the foliage of which assume somewhat small and delicate appearance. This delicacy of the foliage may be due to the influence of genetic substances producing variegation. In the green sports, the genetic condition for chlorophyll production is recovered, and the branches, which live upon the delicate, variegated form, revert to the typical foliage of *Ch. pisifera* var. *plumosa*. Another variegated form of this variety, on which I failed to observe green sports, has, however, quite typical foliage. The dissimilarity in the type of foliage between these two variegated forms probably is caused by the difference of a genetic condition or genotype, by which the variegation is produced. Green sports make generally

vigorous growth compared with variegated, and they are wont to overcome the variegated stocks in growth. The undergrowth of the variegated branches is partly due to their genetic component by which the variegation is manifested, and partly to the frequent occurrence of the albinotic branchlets.

The color and growing habit of the albinotic branches on the variegated forms of the not-common type in various plants are not the same and sometimes very different, owing to the different nature of the factors or the unstable green plastids contained in the cells of the variegated forms at issue.

V

The habitual plastid mutability in the Hordeum type is due to the special quality of a definite factor and also due to a peculiarity of the plastid itself in the Oryza type. In both cases, the frequency of plastid mutability is affected by the influence of ontogenical stages of the plants. In barley the green hybrids carrying the GG' constitution give progenies containing green and variegated individuals at a 3:1 ratio, with no albinos. The dominance of the factor G, therefore, is complete. In addition to this, the plastid mutation does not seem to occur in gametogenesis or in the course of the primitive somatic development, and we have no chance of obtaining albino seedlings in the progeny of such heterozygous greens. The factor G' causes the frequent production of white plastids through the transformation of $g \rightarrow w$, and the factor itself is highly constant, so we have no green individuals among the progeny of the variegated barley in question. In rice, on the contrary, the green individuals appear almost habitually in the progeny of the variegated form of the "unusual" type. This is due to the change of the plastid itself from g' to g, but no factor concerns itself with this event. The frequent alternation of plastids occurs in two directions, $g' \rightarrow w$ and $g' \rightarrow g$, but these two differ in the frequency of occurrence and in the period at which the plastid mutation takes place. The difference in the latter suggests a delicate response of the plastids to the ontogenical environment of the plants in their transformation. The old stems of the variegated variety of *Juniperus chinensis* sometimes give buds which, however, are purely or almost white in the majority of cases, so far as my observation was made. The shooting branches of the variegated *Serissa foetida*, the quality of which is considered to be not common in its variegation, generally bear leaves with small white patches nearly green, while the lateral branches frequently are purely or almost white, especially with the plant

cultivated in a pot. The facts above cited show the delicate response of the plastids as to their tranforming frequency in plant growth. KONDÔ, TAKEDA and FUJIMOTO's observation on mowed stocks of the variegated rice of the "unusual" type gives another confirmation of this relation. Their experiments revealed that "aus diesen Wurzelstocken trieben neue Pflanzen, und die Verfasser haben beobachlet, dasz aus den Wurzelstocken der gestreiften Pflanzen jedesmal in noch höherem Grade weissgestreifte . . . . hervorkamen" (loc. cit. p. 312). The frequent occurrence of white or heavily variegated branches on the variegated plants of the "unusual" type may prove that simultaneous or successive plastid mutation takes place in the rudimentary cells of buds. This happening sustains my postulate as to the origin of the "unusual" type of rice with variegation, as tentatively suggested on a previous page.

Generally speaking, the segregation of albinos among a progeny propagated by the self-fertilization of heterozygous or variegated plants can be summed up as to their genetic types as follows:

I. Mendelian case (figure 3-A).
II. Non-Mendelian case.
    1. Ordinary form (figure 3-B).
    2. Not-ordinary form.
        a. Hordeum type (figure 3-C)
        b. Oryza type (figure 3-D)

In Mendelian segregation the albinism is due to an albino factor, which affecting the plastids make them outwardly colorless. The essential quality of albinotic plastids, however, is perfectly normal. Figure 3-A shows diagrammatically such a relation. The albinism of the ordinary form of non-Mendelian inheritance is due to the massive segregation of white plastids which character is manifested by their own abnormal quality and has no concern with any chlorophyll factors in their manifestation. Figure 3-B represents this relation and the origin of white and green segregates. The albinism of the "special"-typed barley is the same expression as that of the ordinary non-Mendelian case, due to the manifestation of the *w* plastids. But the cause of the successive occurrence of the white plastids is due to the factor *G'*, which at times alters the essential quality of plastids from *g* to *w*. Figure 3-C is drawn to demonstrate this relation. In the Oryza type the albinism is also due to the manifestation of the *w* plastids, the cause of the alteration, however, being attributable to the *g'* plastids themselves which may be phenotypically green, but lacking full constancy. The alteration of the plastids, therefore, occurs quite out of control of the genetic factors but

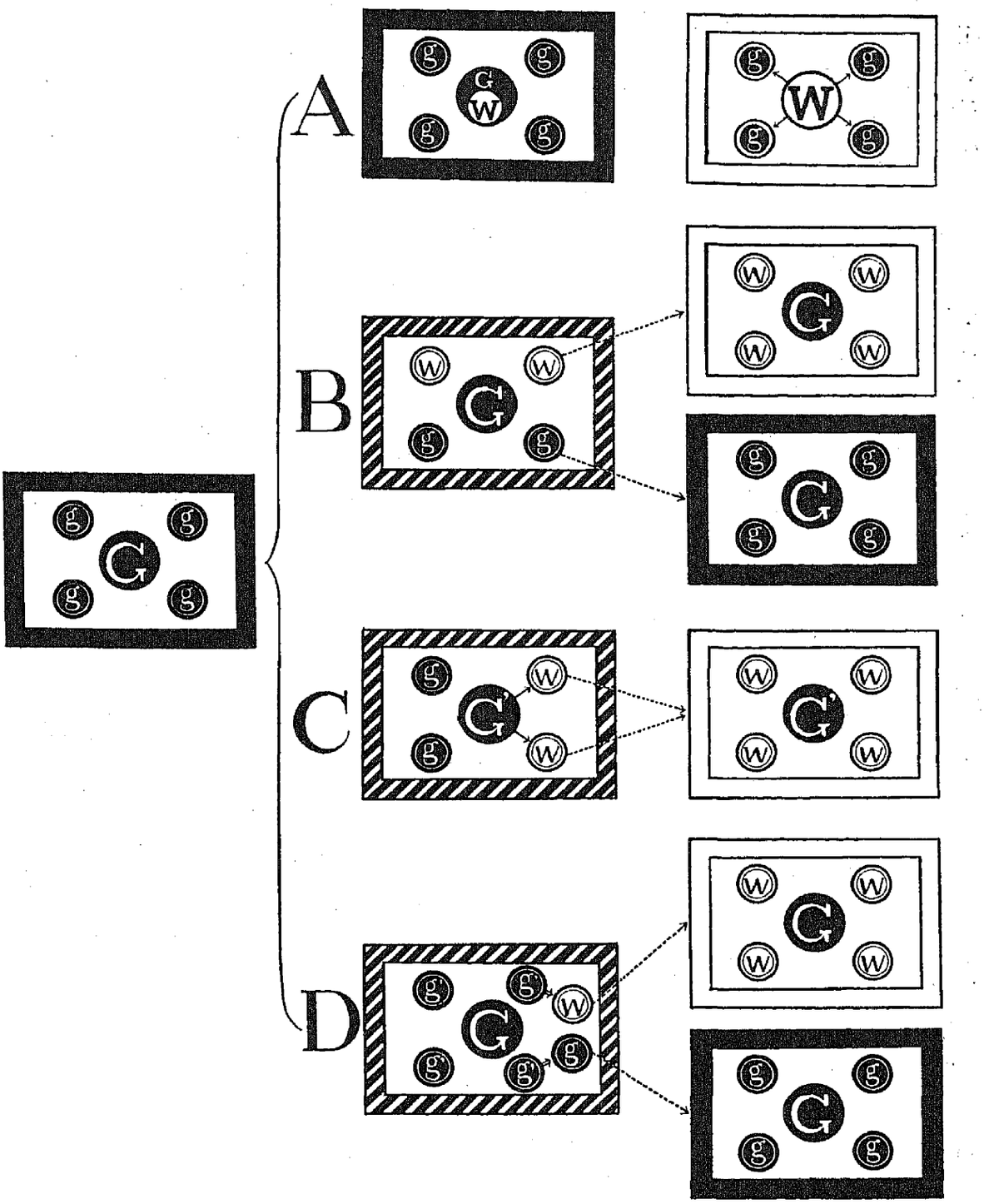


FIGURE 3.—A *schema* showing the genetic types of plastid inheritance, in which albino seedlings are segregated. A and B, Mendelian and ordinary non-Mendelian cases respectively; C, Hordeum type of the "special" form; and D, Oryza type of the "unusual" form. The left square shows the normal green condition. The squares in the center of the figure represent the variegated condition (heterozygote in A which is the Mendelian case). The squares to the right show the albinotic and the green plants sorted out from the respective central squares. Each square shows the model of a cell which contains one nucleus (the large central circle) and four plastids (small peripheral circles). The frame of the cell represents its color, the solid being that of a green cell; the barred that of a variegated cell; and the blank that of a white cell. The genotype of the cell is shown by the color and symbol given in its nucleus. The color of the rim of the plastid indicates its character, while the color and symbol of the center show its real quality.

directly from g' to w. The green individuals among the progeny of the variegated rice in question owe their origin to a similar process; namely, they originated by $g' \rightarrow g$. See figure 3-D, which shows these relations diagrammatically.

VI

The albinotic plastids with albino property usually have a fair constancy as to their quality, and the white cells propagate into purely white tissues or organs by their successive divisions. The common forms of the chlorophyll periclinals and reversals have purely white or yellow skins or cores, which character is non-Mendelain in inheritance, due to the constancy of the quality of the plastids contained in the cells. In some less-known chimaera plants, such as the periclinal variety of *Gardenia florida*, the white skin, which covers the green core, has green ticks and patches, the white tissues being never pure in color. CHITTENDEN (1925) published some interesting results gained in his experimental series of the chimaerical plants, Hydrangea and Pelargonium The variegated forms, on which he made genetical and anatomical studies, contain some complicated chimaeras analogous to the variegated Gardenia above cited. In Hydrangea, he observed three variegated forms,[2] which, according to his denomination, are Types A,[3] B and C, the latter

[2] The common white-over-green form is found in our Hydrangeas, but it was not present in CHITTENDEN'S collection.

[3] To cite CHITTENDEN'S statement, this type "has the stems and the centres of the foliar organs solid white; the latter have a broad margin of solid green. This form, though frequently producing sports wholly green, shows otherwise great regularity of distribution in the type foliage" (loc. cit., p. 44). Such a sort of variegation or "peripheral" chimaera witnessed in some garden plants, is, according to my opinion, probably nothing but a periclinal, in which the yellow or white core seems to contain a toxin that affects the healthy plastids of some outer green tissue by assuming temporarily yellow or white color. The leaf margin of the variegated form, however, does not receive the influence of the toxin secreted by the core, and for that reason the leaf is marked with a green-and-yellow or green-and-white peripheral pattern. This view will explain quite satisfactorily the production of bud-sports, the behavior of which run remarkable parallel with the common periclinals. Among CHITTENDEN'S zonal Pelargoniums, Freak of Nature and Happy Thought seem to be modified periclinals of the green-over-white or green-over-yellow type, in which the white or yellow core affect the green skin at their outer surfaces on both sides of the leaves, resulting in peripheral chimaeras. Chittenden, however, seems not to agree on this view, as he states that "Happy Thought is evidently not a periclinal, for the central *aurea* tissue of the leaves is not covered by any other" (loc. cit., p. 51). This question will be solved by the assumption of a toxic substance, which affects the green plastids in dissolving their chlorophyll, secreted by the albinotic cells or plastids. If these chimaerical forms are Type A of Hydrangea and Freak of Nature and Happy Thought of Pelargonium, with which CHITTENDEN made breeding experiments, they should produce results like those expected from the breeding of the green-over-white or green-over-yellow periclinals. Actually CHITTENDEN'S description of the breeding aspect of the A-typed Hydrangea runs: "This green marginate Type A×green (Type D) throws only

two of which will be treated in this text. Type B[4] is a periclinal with green core which is covered by white skin containing green patches. According to him the breeding aspect of this form is as follows: "When a B Type is fertilized by a green Type D male, it gives both white and green seedlings; when, however, it is used as a male on a green Type D female, only green seedlings results" (loc. cit. p. 44). CHITTENDEN's Type C, however, is a periclinal with green core covered by white skin containing yellow patches instead of green ones.[5] His breeding experiment gave the result that "When fertilized with pollen of a green (Type D), non-viable seedlings, some yellow and some white, result, whereas in the reciprocal cross, only green seedlings are produced" (p. 45). These and other results with Hydrangea led him to conclude that " . . . . the seedlings, regardless of chlorophyll distribution in the male parent, reproduce only the types composing the actual leaf margin of the female." If so how can we understand the origin of green or yellow patches in white tissues? In my opinion, they are probably due to mutations of the albinotic plastids to the green ones, which distribute into massive cells after cell multiplication. The quality of the albinotic plastids contained in the white tissues of these periclinals is not manifested by nuclear substances, but due to the plastids themselves. The mutating green plastids may be induced by the alteration in the quality of the plastids. According to CHITTENDEN, Freak of Nature, whose stems, leaf-stalks and centres of leaves are white, produces white branches having minute green ticks at times on the margins of the stipules and rarely on the

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green seedlings" (p. 44). By selfing the flowers of Freak of Nature he obtained eight seedlings, which were purely green. These results quite satisfy my view. Happy Thought gave segregating progeny in his examination, but, in my opinion, this was induced by the heterozygous genotype of the green skin of the variety. ROTH's experiments (1927) happened to prove the impure genotype of Happy Thought. So the conspicuous segregation does not contradict the periclinal view above proposed. The fact that the adventitious buds formed on the roots of Freak of Nature were always white in color will give an additional confirmation for the hypothesis, according to which the variety consists of periclinal mosaic tissues.

[4] CHITTENDEN states: "This is a white-over-green periclinal, but differs from ordinary periclinal types in having lobes of solid green, without a white skin, irregularly distributed along the margin of the leaves. These lobes occur as outgrowths from the leaf margin. They project from the general contour of the leaf, and their growth is more ample than that of corresponding parts covered by the white skin. Sometimes such lobes are few, but generally several are borne by each leaf. The marginal lobes are usually separated by a white interval from the general body of the green core, and, though seeming occasionally to touch the inner green, they are separate developments, distinct from the core" (loc. cit., p. 44).

[5] According to him, "This form is similar in chlorophyll distribution to Type B, but the lobes consist of pale yellow instead of green tissue. Here also the lobes are areas of rapid growth, and all degrees of lobing occur" (loc. cit., p. 44).

limbs of the leaves. His statement is—that "From such a branch, two wholly green seedlings were once raised; presumably they traced their origin to such a—potentially—green area" (p. 49). I regard the green ticks on the white branches to be due to their mutating origin. Golden Brilliantissima, one of periclinal Pelargoniums, whose behavior was examined by CHITTENDEN, is a form similar to Type B of his Hydrangea. From this form he obtained seven seedlings, all yellow in color, though white ones also may be expected to appear. The absence of white seedlings may possibly not be an incidental result, as he states: "Golden Brilliantissima sets seed badly; a large number of pollinations only produced 156 doubtful-looking seeds, of which 7 germinated" (p. 52). According to the experiment made by CLAUSEN (1927), the seeds of Viola. containing white embryos, do not germinate. At present, however, we cannot draw any conclusion beyond a suggestive statement, and the solution of the problem can confirm a result of further experiments.

CONCLUSION

The inheritance of variegation of the ordinary non-Mendelian type in both barley and rice is closely connected with their particularly coarse pattern of variegation. It is suggested that variegation in these cases is due to sporadic mutation of the plastids themselves, which change from green to albinotic. When the abnormal plastids are later propagated and distributed in the tissues through cell multiplication variegated or albinotic bud-sports are produced. The factorial mutations are generally put into two categories of sporadic and habitual occurrences (IMAI 1927), and likewise the plastid mutations are not confined to sporadic occurrences.

Sô (1921) studied thoroughly the genetics of a finely variegated variety of barley and cleared up its special behavior. The variegated character is transmitted as a simple recessive to the green. The white stripes on the variegated foliage, however, are due to the distribution of white plastids, which have changed their own quality permanently by plastid mutation in the presence of certain recessive genetic factors. The plastid mutation in this case occurs quite habitually and it results in a fine pattern of variegation in this variety. The characteristic of the recessive factor which is contained in the cells of this variety, therefore, is that it alters at times the essential quality of some of the plastids from normal green to colorless. In this case, the plastid mutation occurs so frequently in the course of the development of the plant body that not a single individual or even a leaf or an ear, which is free from the variability or

self-green, is produced. This is interpreted to mean that the variegated character is transmitted itself as a simple Mendelian recessive to the green. The transmission of the white plastids, however, is maternal and does not follow Mendelian rules. So far as I can judge, the mutability of the plastid is not distributed over the whole cycle of the plant development, but the mutable period begins at a later embryonic stage and ceases in later somatogenesis. This assumption explains the particular points of this variegation. The genetics of this variegated barley, therefore, cannot be simply comparable with the ordinary variegation, the case being "special" in its behavior.

The variegated races of rice studied by KONDÔ, TAKEDA and FUJIMOTO (1927) are grouped under two classes in regard to their hereditary type: In one the variegateds reappear in a large proportion of the progeny, in the other practically no variegateds are present but segregation produces green and white seedlings. The latter is identified with the ordinary non-Mendelian case. In the former case, they obtained white, variegated and green progenies in the proportion of 64.5 percent, 30.8 percent and 4.8 percent respectively. The green plants breed invariably true to the type, whereas their variegated sisters repeat the previous performance. The variegation of rice is transmitted maternally in hybridization with green, and the variegated F_1 gives white, variegated and green F_2 segregates in roughly the same proportion as when the variegated plants are self-propagated. They, however, regarded all their cases equally as ordinary non-Mendelian inheritance. In my opinion, the present case is of a not-common form of non-Mendelian inheritance, that is, the plastid mutation occurs quite habitually like the variegated barley of the "special" type. The habitual plastid mutation is not due to any genetic factors contained in nucleus, but to the special quality of plastids themselves contained in the cells of this variegated rice. The plastids alter their quality from green to white by their own instability. The plastid mutation in this case, as in the variegated barley of the "special" type, seems to begin in the late embryonic stage and to cease in the late somatogenesis, including gametogenesis. The appearance of green plants among the progeny of the variegated rice of the "unusual" form is, in my opinion, in accordance with the plastid mutation of the unstable plastids to their non-mutable state. Before the appearance of KONDÔ and his collaborators' paper, TAKEZAKI (1922) published the similar data with variegated rice.

So far as I can judge, there are not a few variegated plants which may be classified under the not-common form in their genetic behavior, among which I have listed several coniferous plants. The plastid mutation

occurs habitually from green to colorless in these plants. Sometimes green branches, due to mutation, are produced on some of these variegated varieties.

The less-known periclinal chimaera plants which have green cores covered by white skins containing green patches, which CHITTENDEN (1925) studied, are interesting material for an investigation of plastid mutation. According to my view, the origination of green or yellow ticks in the white tissues of his chimaera plants may be due to the plastid mutation, occurring quite habitually, from white to green or to yellow.

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